"ANTIDIABETIC ACTIVITY AND TOXICITY STUDIES OF ETHANOLIC EXTRACT OF *POLIALTHIA LONGIFOLIA ROOTS*"

Dissertation Work Submitted to

The Tamilnadu Dr. M. G. R Medical University, Chennai

in partial fulfillment for the award of degree of

MASTER OF PHARMACY

IN

(PHARMACOLOGY)

Submitted by

Mr. AKHIL RAJ, B. Pharm.,
Reg. No: 261425661

Under the guidance of

Dr. D. BENITO JOHNSON, M. Pharm., Ph. D.,
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APRIL-2016

DEPARTMENT OF PHARMACOLOGY
RVS COLLEGE OF PHARMACEUTICAL SCIENCES,
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TAMILNADU

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Certificate

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INTERNAL EXAMINER **EXTERNAL EXAMINER**

Place:

Date:

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Mr. AKHIL RAJ

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ALAT	Alanine amino transferase
ALP	Alkaline phosphatase
ALT	Alanine transaminase
Asp	Aspartate
AST	Aspartate transaminase
ATP	Adenosine triphosphate
STZ	Streptozotocin
BUN	Blood urea nitrogen
ICFE	Ixora Coccinea Flower Extracts
CHF	Congestive heart failure
g	Grams

GIT	Gastro intestinal tract
IU/L	International units/Liter
HDL	High density lipids
LDL	Low density lipid
VLDL	Very low density lipids
TG	Triglycerides
TC	Total cholesterol
WHO	World Health Organization
OECD	Organization for economic corporation development
ICH	International cooperation FOR Harmonization process
EPLR	Ethanol extract of *Polialthia longifolia.*

1. INTRODUCTION

DIABETIC MELLITUS

Diabetic millets[1] often referred to simply as diabetes (Ancient Greek: diabetes" to pass through [urine]") is a syndrome of disorder metabolism, usually due to a combination of hereditary and environment causes, resulting in abnormally high blood sugar level (hyperglycemia).Diabetes is described as starvation in the midst of plenty. Because the body will have high amount of glucose level but the cells are incapable of consume it because of osmotic difference Insulin is a hormone produced in the pancreas, which enable body cells to absorb glucose, to turn into energy. If the body cells cannot absorb the glucose, the glucose accumulates in the blood (hyperglycemia), leading to acute metabolic complications including keto acidosis and in the long term contribute to chronic micro-vascular complications.

TYPES OF DIABETES MELLITUS[2-4]

The type of diabetes is based on the presumed etiology they are

1. Type 1 diabètes or Insulin dependent diabetes mellitus (IDDM)
2. Type 2 diabètes or Non Insulin dependent diabetes mellitus (NIDDM)
3. Gestational Diabetes (Pregnancy diabetic).

1. TYPE 1 DIABETES

Insulin-dependent (Type I) diabetes mellitus is a chronic disease characterized by hyperglycemia, impaired metabolism and storage of important nutrients, evidence of autoimmunity, and long-term vascular and neurologic complications.

Insulin secretary function is limited. Cell membrane binding is not primarily involved. The goal of treatment is to relieve symptoms and to achieve blood glucose levels as close to normal as possible without severe hypoglycemia. However, even with education and self-monitoring of the blood glucose level, attaining recommended target values (plasma glucose level less than 8.0 mmol/L before main meals for adults) remains difficult.

. Therapy with one or two injections per day of mixed short-acting or intermediate-acting insulin preparations is a compromise between convenience and the potential for achieving target plasma glucose levels.

Intensive insulin therapy with multiple daily injections or continuous infusion with an insulin pump improves mean glycated hemoglobin levels; however, it increases rates of severe hypoglycemia and has not been shown to decrease the incidence of clinically significant renal, retinal or neurologic dysfunction. Future prospects include automated techniques of insulin delivery, immunosuppressant to preserve endogenous insulin secretion and islet transplantation.

Type 1 diabetes (IDDM) is characterized by loss of the insulin producing beta cells of the islet of Langerhans in the pancreas leading deficiency. In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required. Type 1 diabetes is usually diagnosed during childhood or early adolescence and it affects about 1 in every 600 children.

It has two forms:

Immune Mediated Diabetes Mellitus**:** Results from a cellular mediated autoimmune destruction of the beta cells of the pancreas.

Idiopathic Diabetes Mellitus**:** Refer to forms of the disease that have unknown etiologies.

The majority of diabetes 1 is of the immune medicated in nature, where beta cells loss is a T cell mediated auto immune attack.

SYMPTOMS OF TYPE 1 DIABETIC

Type 1 diabetes signs and symptoms can come on quickly and may include:

- Increased thirst and frequent urination
- Extreme hunger
- Weight loss
- Fatigue
- Blurred vision

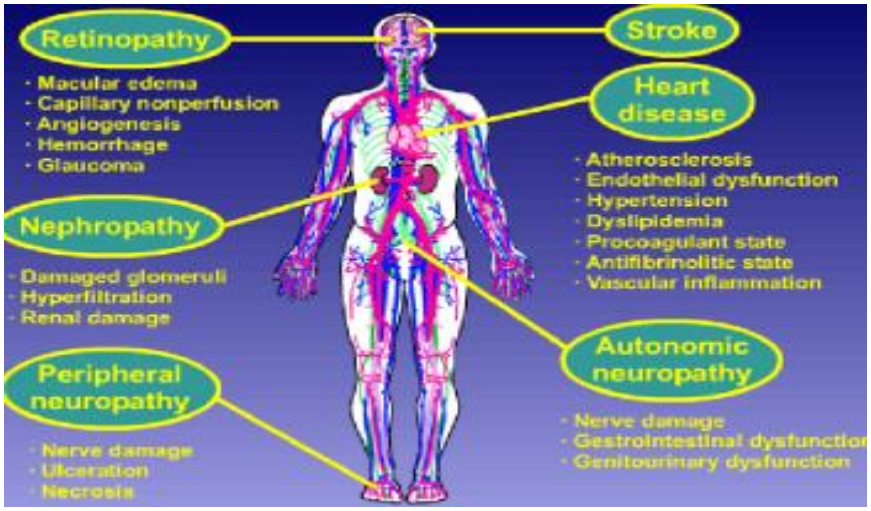
CAUSES OF TYPE 1 DIABETIC.

The exact cause of type 1 diabetes is unknown. In most people with type 1 diabetes, the body's own immune system which normally fights harmful bacteria and viruses mistakenly destroys the insulin-producing (islet) cells in the pancreas. Genetics may play a role in this process, and exposure to certain viruses may trigger the disease.

1) **A family history.** Anyone with a parent or sibling with type 1 diabetes has a slightly increased risk of developing the condition.
2) **Genetics.** The presence of certain genes indicates an increased risk of developing type 1 diabetes. In some cases — usually through a clinical trial genetic testing can be done to determine if someone who has a family history of type 1 diabetes is at increased risk of developing the condition.
3) **Geography.** The incidence of type 1 diabetes tends to increase as you travel away from the equator. People living in Finland and Sardinia have the highest incidence of type 1 diabetes — about two to three times higher than rates in the United States and 400 times that of people living in Venezuela
4) **Viral exposure.** Exposure to Epstein-Barr virus, coxsackievirus, mumps virus or cytomegalovirus may trigger the autoimmune destruction of the islet cells, or the virus may directly infect the islet cells.
5) **Early vitamin D.** Research suggests that vitamin D may be protective against type 1 diabetes. However, early drinking of cow's milk — a common source of vitamin D — has been linked to an increased risk of type 1 diabetes.
6) **Other dietary factors.** Omega-3 fatty acids may offer some protection against type 1 diabetes. Drinking water that contains nitrates may increase the risk. Consuming dairy products, particularly cow's milk, may increase infants' risk of the disease. Additionally, the timing of the introduction of cereal into a baby's diet may affect risk. One clinical trial found that between ages 3 and 7 months appears to be the optimal time for introducing cereal.

COMPLICATIONS OF TYPE 1 DIABETIC MELLITUS

Figure 1: complication of diabetic mellitus[5]



Type 1 diabetes can affect major organs in your body, including heart, blood vessels, nerves, eyes and kidneys. Keeping the blood sugar level close to normal most of the time can dramatically reduce the risk of many complications.

Long-term complications of type 1 diabetes develop gradually, over years. The earlier you develop diabetes — and the less controlled your blood sugar the higher the risk of complications. Eventually, diabetes complications may be disabling or even life-threatening.

1) **Heart and blood vessel disease.** Diabetes dramatically increases your risk of various cardiovascular problems, including coronary artery disease with chest pain (angina), heart attack, stroke, narrowing of the arteries (atherosclerosis) and high blood pressure.
2) **Nerve damage (neuropathy).** Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in the legs. This can cause tingling, numbness, burning or pain that usually begins at the tips of the toes or fingers and gradually spreads upward. Poorly controlled blood sugar could cause you to eventually lose all sense of feeling in the affected limbs. Damage to the nerves that affect the gastrointestinal tract can cause problems with nausea, vomiting, diarrhea or constipation. For men, erectile dysfunction may be an issue.

3) **Kidney damage (nephropathy).** The kidneys contain millions of tiny blood vessel clusters that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end-stage kidney disease, which requires dialysis or a kidney transplant.
4) **Eye damage.** Diabetes can damage the blood vessels of the retina (diabetic retinopathy), potentially leading to blindness. Diabetes also increases the risk of other serious vision conditions, such as cataracts and glaucoma.
5) **Foot damage.** Nerve damage in the feet or poor blood flow to the feet increases the risk of various foot complications. Left untreated, cuts and blisters can become serious infections. Severe damage might require toe, foot or even leg amputation.
6) **Skin and mouth conditions.** Diabetes may leave you more susceptible to skin problems, including bacterial and fungal infections. Gum infections also may be a concern, especially if you have a history of poor dental hygiene.
7) **Osteoporosis.** Diabetes may lead to lower than normal bone mineral density, increasing your risk of osteoporosis.
8) **Pregnancy complications.** High blood sugar levels can be dangerous for both the mother and the baby. The risk of miscarriage, stillbirth and birth defects are increased when diabetes isn't well controlled. For the mother, diabetes increases the risk of diabetic ketoacidosis, diabetic eye problems (retinopathy), pregnancy-induced high blood pressure and preeclampsia.
9) **Hearing problems.** Hearing impairments occur more often in people with diabetes.

TREATMENT FOR TYPE 1 DIABETES

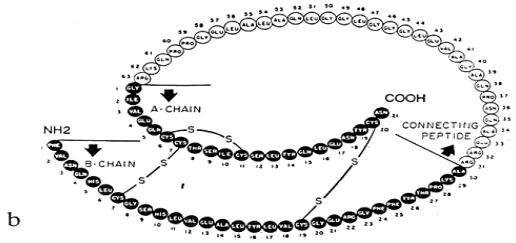
- Exercising regularly and maintaining a healthy weight
- Eating healthy foods
- Monitoring blood sugar
- Taking insulin

The goal is to keep the blood sugar level as close to normal as possible to delay or prevent complications. Although there are exceptions, generally, the goal is to keep your daytime blood sugar levels before meals between **80 and 120 mg/dL** (4.4 to 6.7 mol/L) and your bedtime numbers between **100 and 140 mg/dL** (5.6 to 7.8 mol/L).

INSULIN

Insulin is a peptide hormone, produced by the beta cells of pancreas and is central to regulate the blood glucose level in the body. Like most of the other hormones, insulin is a protein comprising of 2 polypeptide chains A (with 21 amino acid residues and B (with 30 amino acid residues) [Fig. 2]. Chains A and B are linked by disulphide bridges. In addition A-chain contains an intra-chain disulphide bridge linking residue 6 and 11. The structure of insulin is shown in the fi gure 1 below. C-chain, which connect A and B chains is liberated along with insulin after breakdown of proinsulin. Insulin monomers aggregate to form dimers and hexamers[5] .Zn hexamer is composed of three insulin dimmers associated in threefold symmetrical pattern.Patient with type 1 diabetic insulin cannot be secreted in their body (pancreas). So it (insulin) must be injected externally.

Figure 2 **Structure Of Insulin**[6]



b

CELLULAR ACTIONS OF INSULIN [7]

Key insulin target tissues for regulation of glucose homeostasis are liver, muscle, and fat, but insulin also exerts potent regulatory effects on other cell types. Insulin stimulates intracellular use and storage of glucose, amino acids, and fatty acids and inhibits catabolic processes such as the breakdown of glycogen, fat, and protein. It does this by

stimulating the transport of substrates and ions into cells, promoting the translocation of proteins between cellular compartments, activating and inactivating specific enzymes,

and changing the amounts of proteins by altering the rates of transcription and mRNA translation.

Figiure 3:Action of insulin[8]

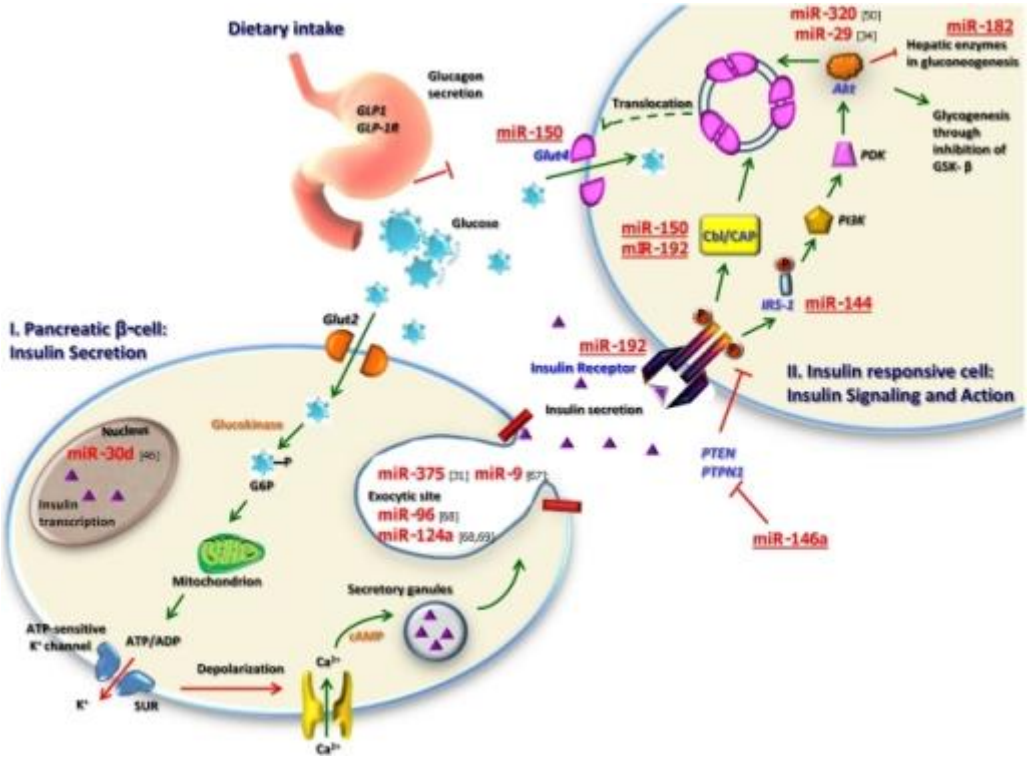


Table: 1 classification of insulin preparation[9-12]

Type of Insulin	Brand Name	Generic Name	Onset	Peak	Duration
Rapid-acting	NovoLog	Insulin aspart	15 minutes	30 to 90 minutes	3 to 5 hours
	Apidra	Insulin glulisine	15 minutes	30 to 90 minutes	3 to 5 hours
	Humalog	Insulin lispro	15 minutes	30 to 90 minutes	3 to 5 hours
Short-acting	Humulin R	Regular (R)	30 to 60 minutes	2 to 4 hours	5 to 8 hours
	Novolin R				
Intermediate-acting	Humulin N	NPH (N)	1 to 3 hours	8 hours	12 to 16 hours
	Novolin N				
Long-acting	Levemir	Insulin detemir	1 hour	Peakless	20 to 26 hours
	Lantus	Insulin glargine			
Pre-mixed NPH (intermediate-acting) and regular (short-acting)	Humulin 70/30 Novolin 70/30	70% NPH and 30% regular	30 to 60 minutes	Varies	10 to 16 hours
	Humulin 50/50	50% NPH and 50% regular	30 to 60 minutes	Varies	10 to 16 hours
Pre-mixed insulin lispro protamine suspension (intermediate-acting) and insulin lispro (rapid-acting	Humalog Mix 75/25	75% insulin lispro protamine and 25% insulin lispro	10 to 15 minutes	Varies	10 to 16 hours
	Humalog Mix 50/50	50% insulin lispro protamine and 50% insulin lispro	10 to 15 minutes	Varies	10 to 16 hours
Pre-mixed insulin aspart protamine suspension (intermediate-acting) and insulin aspart (rapid-acting)	NovoLog Mix 70/30	70% insulin aspart protamine and 30% insulin aspart	5 to 15 minutes	Varies	10 to 16 hours

Each insulin has on own

- ✓ Onset
- ✓ Peak
- ✓ Duration time.

- Onset is how soon the insulin starts a lower the blood glucose
- The peak is the time the insulin is working the hardest to lowering the blood glucose level.
- Duration is the time at how long the insulin lasts the length to keeps lowering the blood sugar

The chart shows the different insulin has different onset, duration and peak.

2. TYPE 2 DIABETES[13]

Type 2 diabetes, once known as adult-onset or noninsulin-dependent diabetes, is a chronic condition that affects the way your body metabolizes sugar (glucose), your body's main source of fuel.

With type 2 diabetes, your body either resists the effects of insulin — a hormone that regulates the movement of sugar into your cells — or doesn't produce enough insulin to maintain a normal glucose level. Untreated, type 2 diabetes can be life-threatening.

It is the result of failure to produce sufficient insulin and insulin resistance. Elevated blood glucose levels are managed with reduced food intake, increased physical activity, and eventually oral medications or insulin

SYMPTOMS OF TYPE 2 DIABETES

Type 2 diabetes symptoms may develop slowly

1) Increased thirst and frequent urination
2) Increased hunger.
3) Weight loss.
4) Fatigue

5) Blurred vision.
6) Slow-healing sores or frequent infections.

CAUSES TYPE 2 DIABETES

1) **Weight.** Being overweight is a primary risk factor for type 2 diabetes. The more fatty tissue you have, the more resistant your cells become to insulin.
2) **Fat distribution.** If your body stores fat primarily in your abdomen, your risk of type 2 diabetes is greater than if your body stores fat elsewhere, such as your hips and thighs.
3) **Inactivity.** The less active you are, the greater your risk of type 2 diabetes. Physical activity helps you control your weight, uses up glucose as energy and makes your cells more sensitive to insulin.
4) **Family history.** The risk of type 2 diabetes increases if your parent or sibling has type 2 diabetes.
5) **Race.** Although it's unclear why, people of certain races — including blacks, Hispanics, American Indians and Asian-Americans — are more likely to develop type 2 diabetes than whites are.
6) **Age.** The risk of type 2 diabetes increases as you get older, especially after age 45. That's probably because people tend to exercise less, lose muscle mass and gain weight as they age. But type 2 diabetes is also increasing dramatically among children, adolescents and younger adults.
7) **Prediabetes.** Prediabetes is a condition in which your blood sugar level is higher than normal, but not high enough to be classified as diabetes. Left untreated, prediabetes often progresses to type 2 diabetes.

COMPLAINTS TYPE 2 DIABETES

1) **Heart and blood vessel disease.** Diabetes dramatically increases the risk of various cardiovascular problems, including coronary artery disease with chest pain (angina), heart attack, stroke, narrowing of arteries (atherosclerosis) and high blood pressure. The risk of stroke is two to four times higher for people with diabetes, and the death rate from heart disease

is two to four times higher for people with diabetes than for people without the disease, according to the American Heart Association.

2) **Alzheimer's disease.** Type 2 diabetes may increase the risk of Alzheimer's disease and vascular dementia. The poorer your blood sugar control, the greater the risk appears to be. So what connects the two conditions? One theory is that cardiovascular problems caused by diabetes could contribute to dementia by blocking blood flow to the brain or causing strokes. Other possibilities are that too much insulin in the blood leads to brain-damaging inflammation, or lack of insulin in the brain deprives brain cells of glucose.
3) **Nerve damage (neuropathy).** Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in the legs. This can cause tingling, numbness, burning or pain that usually begins at the tips of the toes or fingers and gradually spreads upward. Poorly controlled blood sugar can eventually cause you to lose all sense of feeling in the affected limbs.
4) **Kidney damage (nephropathy).** The kidneys contain millions of tiny blood vessel clusters that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end-stage kidney disease, requiring dialysis or a kidney transplant.
5) **Foot damage.** Nerve damage in the feet or poor blood flow to the feet increases the risk of various foot complications. Left untreated, cuts and blisters can become serious infections. Severe damage might require toe, foot or even leg amputation.
6) **Skin and mouth conditions.** Diabetes may leave you more susceptible to skin problems, including bacterial and fungal infections. Gum infections also may be a concern, especially if you have a history of poor dental hygiene.

TREATMENT TYPE 2 DIABETES[14-16]

Diabetes mellitus type 2 is a chronic, progressive disease that has nl established cure, but does have well- established which can delay and sometimes which can delay and sometimes avoid most of the formerly inevitable of the condition.

They are two main goals of treatment.

- ✓ Reduction of morality and concomitant morbidity (from assorted diabetic complication
- ✓ Preservation of quality of life.

Oral hypoglycemic drugs are used to maintain the blood glucose level for some patient I have to take both oral drug and with insulin.

ORAL HYPOGLYCEMIC DRUGS.

a. **Sulphonyl ureas –**
   1. First generation
      i. Tolbutamide
      ii. Chlorpropamide
   2. Second generation
      i. Glibenglamide
      ii. Glipizide
      iii. Gliclazide and Glimiperide

b. **Biguanides**
   1. Metformin

c. Meglitinides
   a. Repaglinide
   b. Nateglinide

d. **Thiazolidine diones** –
   a. Rosiglitazone
   b. Pioglitazone

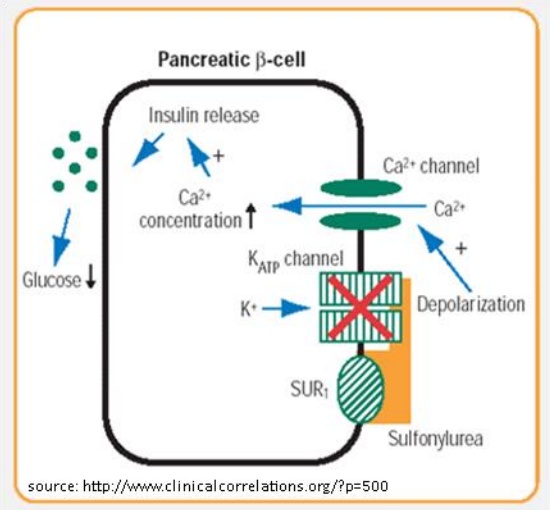
e. **Alpha glucosidase inhibitors**

   Acarbose, Miglitol

Mechanism of action.

Sulfonylurea's bind to (K_{ATP}) channel on the cell membrane of pancreatic this inhibits a tonic, hyperpolarizing efflux of potassium, thus causing the electric potential over the membrane to become more positive. This opens voltage-gated channels. The rises in intracellular calcium leads to increased fusion of granulate with the cell membrane, and therefore increased secretion of (pro) insulin.

Figure 4: mechanism of action suphonylurea's[17]



Tolbutamide

Tolbutamide is a first generation This drug may be used in the management of if diet alone is not effective. Tolbutamide stimulates the secretion of by the. Since the pancreas must synthesize insulin in order for this drug to work, it is not effective in the management of. It is not routinely used due to a higher incidence of adverse effects compared to newer second generation sulfonylurea's, such as it generally has a short duration of action due to its rapid metabolism, and is therefore safe for use in elderly diabetics.

Glimiperide.

The primary mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. In addition, extrapancreatic effects may also play a role in the activity of sulfonylureas such as glimepiride. This is supported by both preclinical and clinical studies demonstrating that glimepiride administration can lead to increased

sensitivity of peripheral tissues to insulin. These findings are consistent with the results of a long-term, randomized, and placebo-controlled trial in which glimiperide therapy improved postprandial insulin/C-peptide responses and overall glycemic control without producing clinically meaningful increases in fasting insulin/C-peptide levels. However, as with other sulfonylureas, the mechanism by which glimepiride lowers blood glucose during long-term administration has not been clearly established.

Glibenglamide.

The drug works by binding to and activating the regulatory subunit of the (K_{ATP}). This inhibition causes cell membrane opening. This results in an increase in intracellular in the and subsequent stimulation insulin release.

After a cerebral ischemic insult is broken and glibenclamide can reach the central nervous system. Glibenclamide has been shown to bind more efficiently to the ischemic hemisphere. Moreover, under ischemic conditions SUR1, the regulatory subunit of the K_{ATP-} and the NC_{Ca-ATP}-channels, is expressed in neurons, atrocities, oligodendrocytes, endothelial cells and by reactive microglia.

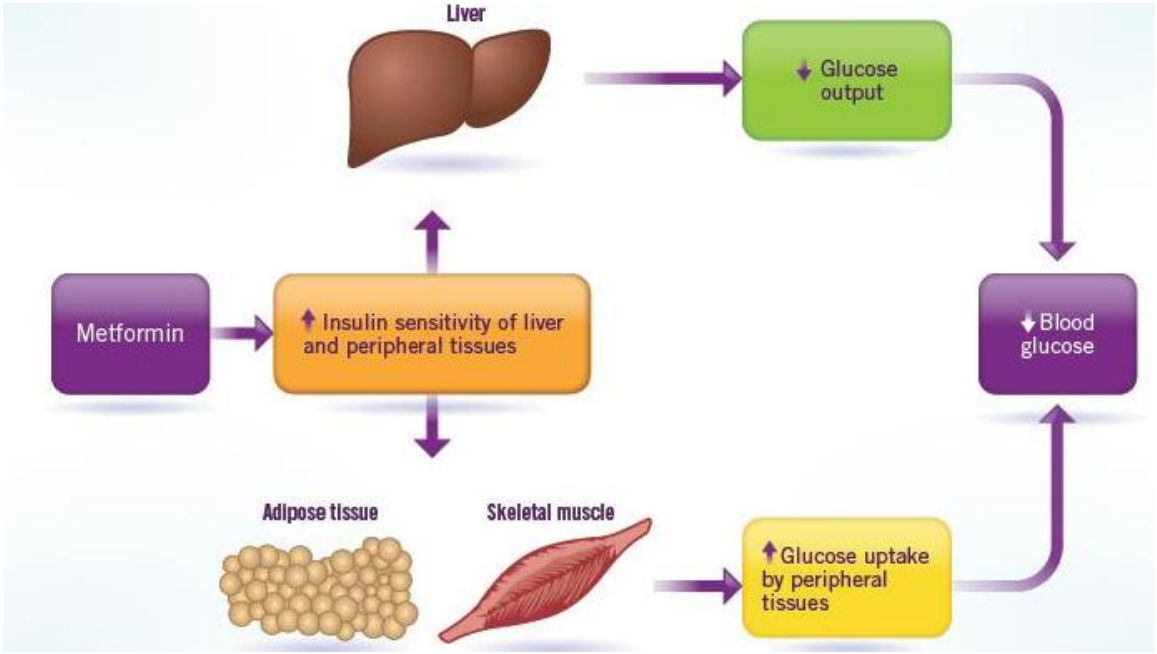
Gliclazide

Gliclazide selectively binds to sulfonylurea receptors on the surface of the pancreatic beta-cells. It was shown to provide cardiovascular protection as it does not bind to sulfonylurea receptors (n the heart This binding effectively closes the K+ ion channels. This decreases the efflux of potassium from the cell which leads to the depolarization of the cell. This causes voltage dependent Ca++ ion channels to open increasing the Ca++ influx. The calcium can then bind to and activate calmodulin which in turn leads to exocytose of insulin vesicles leading to insulin release

Metformin

Metformin activates AMP activated protein kinase (AMPK) a liver enzyme that play an important role in signaling whole body energy balance and the glucose and fats activation of AMPK is required for Metformin inhibitory effects on the production of liver glucose.

Figure 5: Mechanism of action of Metformin[18]



Repaglinide.

Repaglinide acts by stimulating release of insulin from the cells of the islets of pancreas inhibiting ATP-sensitive K+ channels, thereby activating the Ca++ channels with increase in intracellular calcium to release insulin. However, repaglinide acts on a different binding site than the sulphonylureas. Repaglinide is not effective in the absence of functioning beta-cells. Repaglinide increases the amount of insulin released in a natural and physiological pulsatile pattern the activity of repaglinide is dose-dependent. Mean insulin levels begin to rise approximately 1.5 hours after the pre-prandial dose of repaglinide and declines towards baseline levels between meal-time the rapid onset of action and the short duration of hypoglycemic effect of repaglinide makes this agent suitable for pre-prandial administration. The main advantage of pre-prandial administration is that patients can miss or postpone a meal (and the corresponding repaglinide dose) without increasing the risk of hypoglycemia or compromising glycaemic control.

Pioglitazone.

Pioglitazone is an oral drug that reduces the amount of glucose (sugar) in the blood. It is in a class of anti-diabetic drugs called thiazolidinediones that are used in the treatment .The other member in this class is . (Another member of this class, troglitazone or Rezulin, was removed from the market because of .) Patients with type

2 diabetes cannot make enough insulin, and the cells of their body respond less to the insulin that is produced. Since insulin is the hormone that stimulates cells to remove glucose from the blood, the reduced amount of insulin and its reduced effect cause cells to take up less glucose from the blood and the level of glucose in the blood to rise. Pioglitazone often is referred to as an "insulin sensitizer" because it attaches to the insulin receptors on cells throughout the body and causes the cells to become more sensitive (more responsive) to insulin. As a result, more glucose is removed from the blood, and the level of glucose in the blood falls. At least some insulin must be produced by the pancreas in order for pioglitazone to work. Pioglitazone also lowers the level of glucose in the blood by reducing the production and secretion of glucose into the blood by the liver. In addition, pioglitazone may alter the blood concentrations of lipids (fats) in the blood. Specifically, it decreases and increases the "good" (HDL)

Acarbose.

Acarbose inhibits enzymes, specifically, enzymes in the brush border of the small intestines and pancreatic alpha-amylase hydrolyzes complex starches to in the lumen of the small intestine, whereas the membrane-bound intestinal alpha-glucosidase in the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecule.

III) GESTATIONAL DIABETES

This type affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose. Diagnosis of gestational diabetes is made during pregnancy.

The majority of gestational diabetes patients can control their diabetes with exercise and diet. Between 10% to 20% of them will need to take some kind of blood-glucose-controlling medications. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. The baby may be bigger than he/she should be.

COMPLICATIONS GESTATIONAL DIABETES

i. Increased risk of prenatal mortality and morbidity.

ii. Obesity or impaired glucose intolerance in the offspring accompanied by macrosomia

iii. Neural tube defects

iv. Prematurity syndromes

CAUSES GESTATIONAL DIABETES.

Age > 30 years, obesity (BMI > 27.3 kg/m2), family history of diabetes, glycosuria, previous macrosomia, previous congenital malformation, previous stillbirth, past history of Gestational diabetes mellitus.

TESTES FOR DIABETIC

- **Glycated hemoglobin (HbA1C) test.** This blood test indicates your average blood. The term HbA1c refers to Glycated hemoglobin. It develops when hemoglobin a protein within red blood cells that carrier's oxygen throughout the body. Joins in the blood becomes "Glycated" by measuring Glycated hemoglobin (HbA1c), clinicians are able to get an overall picture of what is the average blood sugar level have been over a period of week or month

- .**Random blood sugar test.** A blood sample will be taken at a random time. Blood sugar values are expressed in milligrams per deciliter (mg/dL) or mill moles per liter (mmol/L). Regardless of when you last ate, a random blood sugar level of 200 mg/dL (11.1 mmol/L) or higher suggests diabetes, especially when coupled with any of the signs and symptoms of diabetes, such as frequent urination and extreme thirst.

- **Fasting blood sugar test.** A blood sample will be taken after an overnight fast. A fasting blood sugar level less than 100 mg/dL (5.6 mmol/L) is normal. A fasting blood sugar level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is considered prediabetes. If it's 126 mg/dL (7 mmol/L) or higher on two separate tests, you have diabetes.

DIABETIC INDUCED BY STREPTOZOTOCIN.[19]

Streptozotocin is a toxic glucose analogue that preferentially accumulates in pancreatic beta cells via the GLUT2 glucose transporter. Especially the Streptozotocin inhibits insulin secretion and causes a state of insulin dependent diabetes mellitus. Both effects can be attributed to its specific chemical properties, namely its alkylating potency as with Streptozotocin its beta cell specificity is mainly the result of selective cellular uptake and accumulation.

Beta cells selectivity of Streptozotocin [20]

Streptozotocin is a nitrosourea analogue in which the *N* methyl *N nitrosourea* (MNU) moiety is linked to the carbon 2 of a hexose. The action of Streptozotocin and chemically related alkylating compounds requires their uptake into the cells. Nitrosourea are usually lipophilic and tissue uptake to a plasma membrane in rapid; hour as a result of the hexose substitution, Streptozotocin is less lipophilic. Streptozotocin is selectively accumulated in pancreatic β cells via the low- affinity GLUT2 glucose transporter in the plasma membrane. Thus, insulin-producing cells they do not express this glucose transporter are resistant to Streptozotocin. This observation also explains the greater toxicity of Streptozotocin compared with N-methyl-N-nitrosourea in cells that express GLUT2, even though both substances alkylating DNA to a similar extent. The importance of the GLUT2 glucose transporter in this process is also shown by the observation that Streptozotocin damages other organs expressing this transporter, particularly kidney and liver.

β cells toxicity

It is generally assumed that the toxicity of Streptozotocin is dependent up on the DNA alkylating activity of this methylnitrosourea moiety especially at the 6 position of guanine. The transfer of the methyl group Streptozotocin DNA molecules causes damage, which along a define chain of events, results in the fragmentation of the DNA. Protein glycosylation may be an additional damaging factor. In the attempt to repair DNA polymerase is over stimulated. This diminishes cellar NAD+ and subsequently ATP Ultimately responsible for beta cell death, but it is likely that protein methylation contributed to the functional defects of the beta cells after exposure to Streptozotocin

Inhibition of insulin secretion by Streptozotocin

The effects of Streptozotocin on glucose and insulin homeostasis reflect the toxin induced abnormalities in beta cell function. Initially biosynthesis, glucose induced

insulin secretion and glucose metabolism is all affected. On the other hand Streptozotocin has no immediate direct inhibitory effect upon glucose transport or upon glucose stage of transport or upon glucose phosphorylation by glucokinase. However at later stages of functional beta cells impairment deficiencies in teams of gene expression and protein production lead to the deterioration of both glucose transport and metabolism

Even before the negative effect of mitochondrial DNA and protein alkylation and glycosylation become evident Streptozotocin induced depletion of NAD may result in the inhibition of insulin biosynthesis and secretion later inhibition of glucose induced and amino acid induced insulin secretion as a result of mitochondrial genome become apparent. This impairment is more marked for nutrient that for non nutrient insulin secretion. This interpretation has been confirmed through studies which have shown that pre treatment of isolated pancreatic islet with polymerase inhibitor nicotinamide prevents early inhibition of beta cells function beta cell function during first day after Streptozotocin exposure while long term inhibition of insulin secretion 6 days after Streptozotocin exposure

MECHANISM OF ACTION OF STREPTOZOTOCIN.

STZ

↓

Enter beta cells

↓

Via glucose transporter

↓

Alkylation of DNA

↓

Activity of poly ADP ribosylation

↓

Depletion of NAD and ATP

↓

Enhance ATP dephosphorylation

↓

Super oxide radicals

↓

Hydrogen peroxide free radical

↓

Hydroxyl radicals

↓

Liberate toxic amount of NO

↓

DNA damage

↓

Hyperglycemia and hypoinsulinemia

HERBAL MEDICINE TODAY

In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs as they are generally non-toxic and World Health Organization has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs. Plant derivatives with hypoglycemic properties have been used in folk medicine and traditional healing systems around the world from very ancient time. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem to people[21] .Medicinal plants used to treat hypoglycemic and hyperglycemic conditions are of considerable interest to ethno botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant. There are a large number of plants and natural biomolecules that have been discussed in literature for their antidiabetic effects. For example, plants have been used since ancient times to prevent conditions associated with diabetes [22]. There is a growing interest in herbal remedies, because of their effectiveness, minimal side effects in clinical experience, and relatively low cost. Herbal drugs or their extracts are prescribed widely; even their biologically active compounds are unknown[23] .In many developing countries, traditional medicine, in particular the herbal medicine, is sometimes the only affordable source of healthcare[24] Even the WHO (World Health Organization) approves the use of plant drugs for different diseases, including diabetes mellitus[25] This mini review aims to discuss some key aspects related to the potential use of plants and natural biomolecules for the prophylaxis and treatment of type 2 diabetes as well as the potential mechanisms of action.

In traditional medicine diabetes mellitus is treated with diet, physical exercise and medicinal plants, even though, more than 1200 plants are used around the world in the control of diabetes mellitus and approximately 30% of the traditionally used ant diabetic plants were pharmacologically and chemically investigated. On the other hand, potential hypoglycemic agents have also been detected for more than 100 plants used in ant diabetic therapy. Traditional treatments may provide the valuable clues for the development of new oral

hypoglycemic agents and simple dietary adjuncts. More than 100 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of diabetes, which are effective either separately or in combinations

2.LITERATURE REVIEW

Kashalkar Rhreeajas V et al[26] was carried out the preliminary studies of *P.longifolia* seeds to investigate its potentialities. The preliminary phytochemical evaluation of various extracts indicated that the seeds are rich source of alkaloids, tannins, phenols, flavonoids and carbohydrates. Loss on drying and moisture content experiments was carried out to know the presence of volatile organic matters.

Raja Sidambaram R et al[27] was evaluated the antioxidant activity of seed extracts of *Polyalthia longifolia*. Petroleum ether, chloroform, methanol and aqueous extracts of seeds of *P.longifolia* were prepared and evaluated by preliminary phytochemical screening followed by antioxidant activity using DPPH and FRAP assays. The phytochemical analysis showed the major active compound present in all the four extracts as terpenoids. Among the various extracts methanol and petroleum ether extracts showed good antioxidant activity and aqueous extract showed very low activity in both the antioxidant assays. The study confirmed the antioxidant activity of different seed extracts of *P.longifolia* and can be used for further drug formulation studies.

S Kaviya et al[28] was synthesized the Synthesis of silver nanoparticles (AgNPs) using *Polyalthia longifolia* leaf extract. The reaction is carried out at two different concentrations (10−3M and 10−4 M) of silver nitrate, and the effect of temperature on the synthesis of AgNPs is investigated by stirring at room temperature (25◦C) and at 60◦C. The UV-visible spectra of NPs showed a blue shift with increasing temperature at both concentrations. FT-IR analysis shows that the biomoites played an important role in the reduction of Ag+ ions and the growth of AgNPs. TEM results were utilized for the determination of the size and morphology of nanoparticles. The synthesized silver nanoparticles are found to be highly toxic against Gram-positive bacteria than Gram-negative bacteria.

Prashith Kekuda T.R et al[29] determining antimicrobial activity of leaf and pericarp (ripe and unripe) extracts of *Polyalthia longifolia* . Antibacterial activity

of leaf and pericarp extracts was determined against *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* by Agar well diffusion assay. Antifungal activityof leaf and pericarp extracts was tested against *Sclerotium rolfsii* by Poisoned food technique. The extracts were effective against *S. aureus* to higher extent when compared to Gram negative bacteria. The extracts caused marked inhibition of mycelial growth of *S. rolfsii*. Unripe pericarp extract exhibited marked inhibition of bacteria and fungus when compared to other extracts. Preliminary phytochemical analysis of extracts revealed the presence of flavonoids, tannins, steroids and glycosides in leaf and pericarp extracts. The observed inhibitory potential could be ascribed to the presence of secondary metabolites in the extracts.

Jayaraman Rajangam et al[30] was hepatoprotective and antioxidant potential of methanolic extract of *Polyalthiya longifolia* fruits (MEPL) by *in-vitro* and *in-vivo* methods.In the invitro study, Freshly isolated rat primary hepatocytes and HepG2 cells were exposed with CCl4 along with/without various concentrations of MEPL(125,250,500 μg/kg) and the effects were studied.In the *in-vivo* studies, CCl4 intoxication method was used and aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin and total proteins were estimated and supported with histopathological studies.MEPL (125,250,500 μg/kg) treated animals increased the percentage of viability in both primary hepatocytes ($p<0.001$) and in HepG2 cells ($p<0.01$) where as in the invivo studies,MEPL produced significant hepatoprotective effect by decreasing the elevated serum enzymes, bilirubin, LPO and significantly increased the levels of glutathione (GSH), catalase (CAT) and super oxide dismutase (SOD) .Moreover, *in-vitro* antioxidant studies revealed that MEPL scavenged free radicals and maximum percentage of inhibition was 62% at 800μg/mL.

Aparna Lakshmi et al[31] was evaluated by estimating the blood glucose level, total protein, total cholesterol, creatinine and blood urea and nitrogen in alloxan induced diabetic rats. The wound healing activity property was studied by excision and incision methods.There is a significant decrease in the blood glucose levels from 1 week to 3 week in n-hexane extract, ethyl acetate and methanolic extract treated

groups when compared to the diabetic control group. The n-hexane extract treated group has shown significant increase in the total cholesterol, creatinine and urea levels when compared to the other treated groups and is almost similar to the standard group. In contrast the methanolic extract has brought has brought the total protein level to the normal in diabetic induced rats. In conclusion the present study indicated a significant antidiabetic effect of the methanolic extract of *Polyalthia longifolia* and supports its traditional usage in the control of diabetes. It was found to have strong wound healing property. Further studies are required for the detailed studies in isolation of the compounds and pharmacological investigations of the bark constituents, which possess its own traditional claim.

Yang Chang Wu et al[32] isolated Three new clerodane diterpenes, (4→2)-abeo-cleroda-2,13E-dien-2,14- dioic acid (1), (4→2)-abeo-2,13-diformyl-cleroda-2,13E dien-14-oic acid (2), and 16(R&S)- methoxycleroda-4(18),13-dien-15,16-olide (3), were isolated from the unripe fruit of Polyalthia longifolia var. pendula (Annonaceae) together with five known compounds (4-8). The structures of all isolates were determined by spectroscopic analysis. The anti-inflammatory activity of the isolates was evaluated by testing their inhibitory effect on NO production in LPS-stimulated RAW 264.7 macrophages. Among the isolated compounds, 16-hydroxycleroda-3,13-dien-15,16-olide (6) and 16-oxocleroda-3,13- dien-15-oic acid (7) showed promising NO inhibitory activity at 10 μg/mL, with 81.1% and 86.3%, inhibition, respectively.

3. PLANT PROFILE

.....Polyalthia longifolia[33] *....*

TAXONOMICAL CLASSIFICATION [34]

Table: 2 – Taxonomical classification of *Polyalthia longifolia*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Magnoliales;
Family	Annonaceae
Genus	*Polyalthia*;
Species	*longifolia*

Table: 3 – Vernacular names of *Polyalthia longifolia* [35]

English	Ashoka,
Hindi	Deodari
Tamil	Asogu
Bengali	Debdaru
Malayalam	Aranamaram

Distribution and habitat[36]:

Polyalthia longifolia cv. *pendula* (Annonaceae) is native to the drier regions of India and is locally known as "Ashoka" and is commonly cultivated in India, Pakistan, and Sri Lanka

Description:

The plant grows throughout the tropical and subtropical parts of India up to an altitude of 1500 m. A tall, evergreen, handsome, pyramid-like, columnar, tree: main stem straight, undivided, growing up to 12 m or more. Branches slender, short, about 1-2 m long, glabrous, and pendulous. Leaves alternate, exstipulate, distichous, mildly aromatic, 7.5-23 by 1.5-3.8 cm, shining, glabrous, narrowly lanceolate, tapering to a fine acuminate apex, margin markedly undulate, pinnately veined, leathery or subcoriaceous, shortly petiolate; petiole about 6 mm long. Flowers arise from branches below the leaves, nonfragrant, 2.5-3.5 cm across, yellowish to green, in fascicles or shortly pendunculate umbels; petals 6, 2 seriate, flat, from a broad base, lanceolate, long accumulate, spreading; and sepals 3, broad, short, triangular, the tips reflexed. Stamens many, cuneate; connective truncately dilated beyond the cells. Ovaries indefinite; ovules 1-2; style oblong. Ripe fruits ovoid, 1.8-2 cm long, numerous, stalked, glabrous, 1 seeded; stalk 1.3 cm long, short, glabrous. Seeds smooth, shining. Flowering and fruiting: February-

CHEMICAL CONSITUVENTS

P. longifolia mainly contains diterpenoids,[27] alkaloids, tannins, and mucilage. The chief components of the plant are O-methylbulbocapnine-*N*-oxide (1),

polyfothine (2), *N*-methylnandigerine-*N*-oxide (3), oliveroline-*N*-oxide (4), pendulamine A (5), *N*-pendulamine B (6), 8-oxopolyalthiane (7), 16-oxo-5 (10), 13-halimadien-15-oic acid (8), 16-Oxo-3, 13-clerodadien-15-oic acid (9), 16-hydroxycleroda-3, 13-dien-16, 15-olide

Uses[36]:

Almost all parts of the plant are used in the Indian traditional system of medicine for the treatment of various ailments in human beings. In Ayurveda, particularly, the bark of *P. longifolia* has significant medicinal properties.

4. AIM AND OBJECTIVE

It is divided into following phase-

PHASE I : Taxonomical studies.

- ✓ Collection of plants.
- ✓ Authentication of plants.
- ✓ Powdered the root materialls

PHASE II :Pharmacognostical studies.

- ✓ Successively solvent Extraction.
  - Alcohol (ethanol).
- ✓ Preliminary Phytochemistry screenings.
  - Alkaloids
  - Saponins.
  - Tannins
  - Amino acid
  - Flavonoids
  - Terpenoids
  - Protein
  - Steroids

PHASE III :Pharmacological studies.

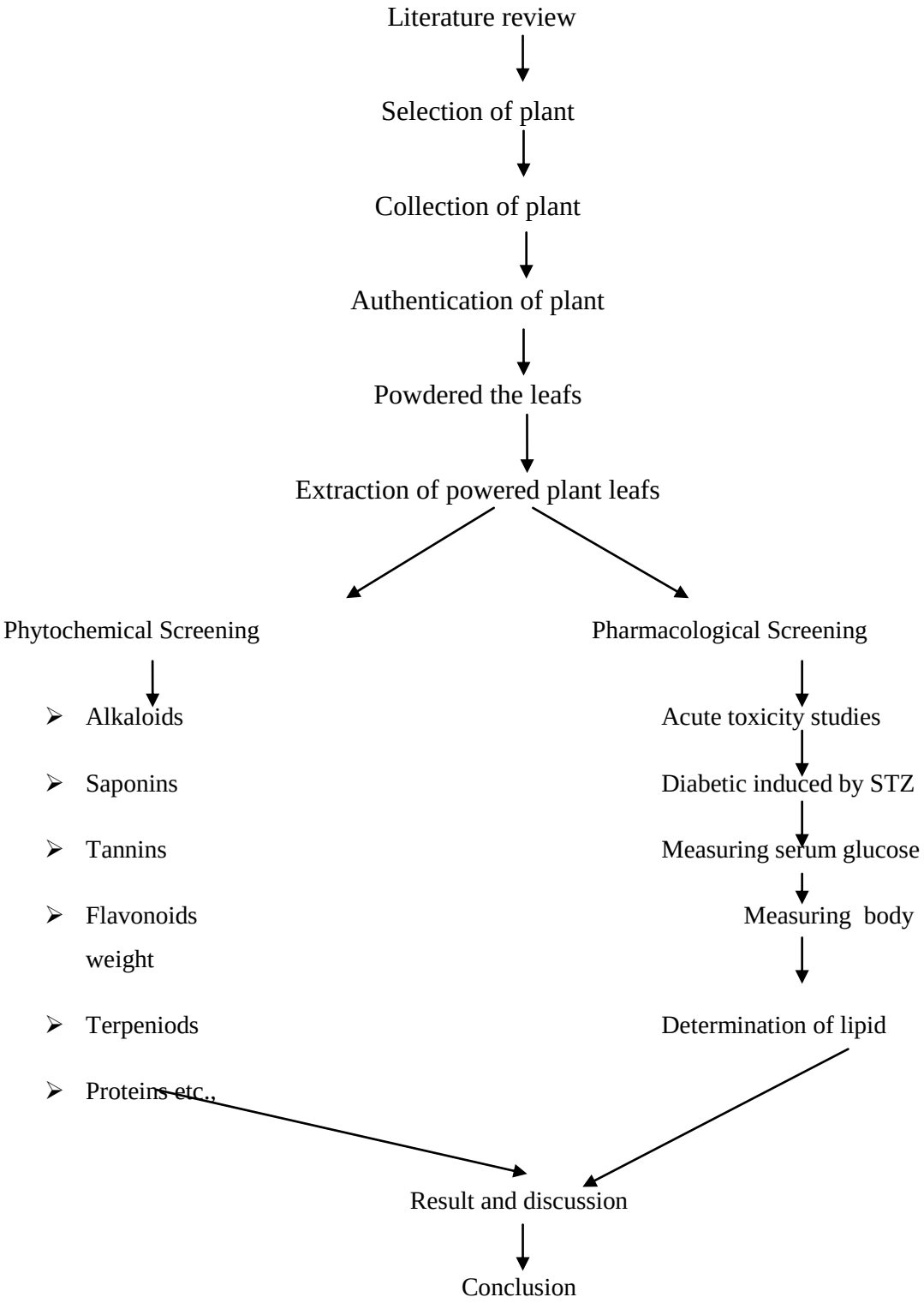
- ✓ Acute toxicity studies (as per OECD guidelines)
- ✓ Induction of diabetic animal
- ✓ Induces diabetic in rats by STZ
- ✓ Measuring the body weight

- ✓ Measuring the water intake
- ✓ Measuring the food intake
- ✓ Determination of lipids
    - o Triglyceride
    - o Total cholesterol
    - o HDL
    - o VLDL

PHASE IV : Result and Documentation.

Evolution of statistical significance result by computer aided program and systemic documentation. Values were presented as mean SEM Data were analyzed using of variance (ANOVA) and group means were compared with Turkey's post hoc Multiple Comparison Test using SPSS software version 17. $P<0.05$ is considered as significant.

PLAN OF WORK


Literature review
Selection of plant
Collection of plant
Authentication of plant
Powdered the leafs
Extraction of powered plant leafs
Phytochemical Screening
Alkaloids
Saponins
Tannins
Flavonoids
Terpeniods
Proteins etc.,
Pharmacological Screening
Acute toxicity studies
Diabetic induced by STZ
Measuring serum glucose
Measuring body weight
Determination of lipid
Result and discussion
Conclusion

5. MATERIALS AND METHOD

Chemicals :

- Streptozotocin.
- Glibenclamide
- Petroleum ether
- Ethanol
- Benzene
- Chloroform
- Studies were carried out in albino rats.

TAXONOMICAL STUDIES.

1) **Collection of plants.**

P. Longifolia was produced from the Botany Central council for Research in Ayurvedia and Siddha Govt of India.

The freshly collected sample were thoroughly cleaned and soaked in fresh water repeatedly to separate mud particles sticking on to plant constituents. The plants constituents collected were cut into small bits of about 2-3 in size. After the leafs of the plants are powdered with a mechanical grinder. This powder was subjected to various studies for which the materials and methods which is presented below.

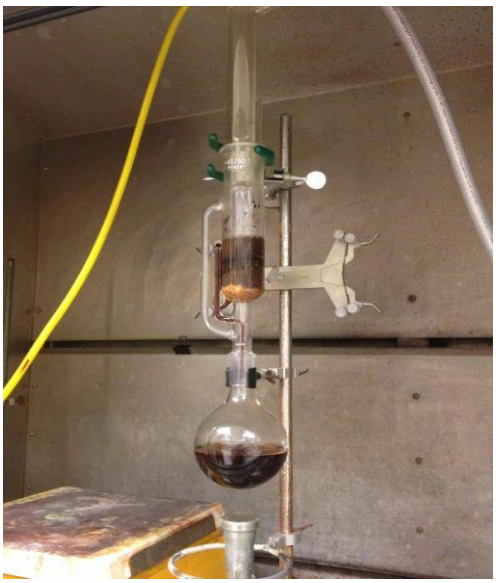
2) Authentication of plant.

The dried whole plant powder of *P. Longifolia Roots* was supplied and authenticated by Chelladurai.v research officer Botany Central council for Research in Ayurvedia and Siddha Govt of India.

PHARMACOGNOSTICAL STUDIES.

Extraction procedure.

Figure 7: Assembly extraction Apparatus



Equal amount of the weighed powder were mixed and placed in the Soxhlet apparatus by using ethanol. From the 500gm of crude powder was extracted with 2.5 liter of ethanol (60 – 80) by continuous hot percolation using Soxhlet apparatus. This can be continued up to 24 hours.. After the extraction process filtered and the Soxhlet is removed the obtained residue is stored in the dissector.

PHYTO CHEMICAL SCREENING[37-39]

The plant may be containing the following compound such as carbohydrate, protein, and lipids. That is utilized as food by man. It also contains the compound like. Tannins, glycosides, alkaloids. Volatiles oils. The compound that is responsible for lots of medicinal properties

TEST FOR CARBOHYDRATES

Molish test

- The sample powdered was added with 1 ml of alpha naptool solution along with conc Sulphuric acid solution in the test tube reddish colour was

produced at the junction between 2 liquid this is shows the presence of carbohydrate.

Fehling test.

To the sample powder was added with both Fehling A and Fehling B solution and placed in the water bath for a sufficient time. This shows the brick red colour. It shows the presence of carbohydrate.

Benedicts test.

To the sample powder add 8 drops of benedicts reagents and voil the sample vigorously for 5 min it shows the red ppt. this shows the presents of carbohydrate.

TEST FOR ALKALOIDS

To the small of stored powder (sample) was taken and add few drops of hydrochloric acid and filtered.

The filtered was tested with various alkaloid agents.,

Mayer's reagents:

To a small of above filter add small quantity of Mayer's reagent to form cream precipitate. This shows the presence of alkaloids.

Dragendorffs reagents

From the above filter add small amount of Dragendorffs reagents it forms a orange brown precipitate. This shows the presents of alkaloids.

TEST FOR FLAVONOIDS

To the filter of the plant extract add 5 ml of dilute ammonia solution and followed by the addition of concentrated sulphuric acid. It forms a yellow colour. It shows extract indicated the presence of flavonoids.

TEST FOR STEROIDS.

Salkowaski test

Few amount of plant extract was mixed with chloroform and the same volume of sulphuric acid is added on it. Cherry red colour was obtain in the chloroform layer. This shows the sample contain steroids.

Libbbermann burchatd test:

The extract is dissolved in 2 ml of chloroform 10 drops of acetic acid and conc. Sulphuric acid were added. Now the solution becomes reddish

colour then it turns to bluish green colour. This shows the plant extraction indicates the presents of steroids.

TEST FOR TANNINS.

From few amount of plant extract is treated with vanillin hydrochloric acid reagent. It forms, pink or red colour due to the formation of phloroglucinol, it indicate the presence of tannins.

TEST FOR PROTEIN.

Mellon's reagents.

Mellon's reagents (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.

Ninhydrin Test.

From the sample solution add 2 drops a freshly prepared 0.2% ninhydrine reagent was added to the extract and heating. Development of blue colour may indicate the presence of peptide, amino acid (PROTEIN).

TEST FOR GLYCOSIDES:

Keller- killani test.

From the small quantity of small powder acetic acid was dissolved and adds few drops of ferric chloride and transferred to the surface of conc Sulphuric acid. At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presents of cardiac glycosides.

TEST FOR SAPONINS.

Foam test:

1 ml of extract solution is diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of Saponins.

PHARMACOLOGICAL STUDIES.

TOXICITY STUDY[40].

ACUTE TOXICITY STUDY

Experimental Protocol

Guideline : OECD-423

CPCSEA Ref. No : IAE1012/c/06/CPCSEA

Test : Limit test

Species : *Rattus norvegicus*

Strain : Albino Wistar rats

Number of animals : 10 animals (5 for each kashayams)

Sex : Female

Initial dose : 5mg/kg

Route of administration : Oral

Duration : 3 hr close observation, followed by 14 days observation

Others : Body weight, mortality status

Parameters : CNS, ANS and behavioral changes

Blood collection : Not needed

Sacrifice : On day 14 after oral administration

Table 4- EXPERIMENTAL DESIGN

GROUP	DOSE (mg/kg)
Group 1	5
Group 2	50
Group 3	300
Group 4	2000

STUDY DESIGN

Selection of Test animal

Female adult Wistar rats of 8-12 weeks are selected. Nulliparous and non-pregnant animals were obtained from the centralized animal house of RVS College of

Pharmaceutical sciences, Sulur and they are acclimatized for holding 1 week prior to dosing.

Housing and feeding conditions

Temperature - As per OECD GUIDELINE-420 the temperature of animal house is maintained at $22^0C \pm 3^0C$.

Humidity - The relative humidity of animal room maintained at 50-60% preferably not exceeds 70% (OECD guidelines-420, 2001). Otherwise there may be chances of developing lesions such as ring tail and food consumption may be increased.

Light – The sequence of light used is 12 hrs light and 12 hrs dark.

Caging – Polypropylene cages with solid bottom and walls. The lids are made up of stainless steel grill which is capable to hold both feed and water.

Feeding condition and feed – Sterile laboratory feed (*ad libitum*) and water daily. The feed used is brown coloured chow diet.

Drug administration

Animals are fasted prior to dosing (food but not water should be withheld for overnight).After that animals are weighed and the test substance administered. The healthy rats has been taken and divided into 4 different groups. Then the petroleum ether extract was dissolved in 0.6% if sodium carboxyl methyl cellulose on equal. The test substance is administered in a single dose by oral gavages, using a curved and ball tipped stainless steel feeding needle.

Clinical observation

All animals were monitored continuously with special attention for 4 hrs after dosing for signs of toxicity. Additional observations are also done for the next 14 days for any other behavioral or clinical signs of toxicity. Weight changes are calculated. At the end of the test animals are weighed and then humanely killed andLD_{50} values are established.

SUB ACUTE TOXICITY STUDY

Experimental Protocol

Guideline	: OECD-407
CPCSEA Ref. No	: 1012/c/06/CPCSEA
Species	: *Rattus norvegicus*
Strain	: Albino Wistar rats
Number of animals	: 10 for each groups

Sex : Male/Female

Route of administration : Oral

Duration : 28 days

No. of blood collection : 2

Duration of blood collection : 0^{th} day, 28^{th} day

Blood collection route : Retro orbital

Sacrifice : After 28 days of oral administration

Body weight recording : weekly intervals

Table 05-EXPERIMENTAL DESIGN FOR SUBACUTE TOXICITY STUDIES

Groups	Dose	No. of animals
Group 1	Control	10(5M+5F)
Group 2	Low dose	10(5M+ 5F)
Group 3	Medium dose	10(5M+ 5F)
Group 4	High dose	10(5M+ 5F)

STUDY DESIGN

Selection of Animals

Male and Female rats were selected and are acclimatized for 5 days prior to the start of study. The females are nulliparous and non-pregnant. At the commencement of study the weight variation of animals used minimal and not exceed ± 20 % of the mean weight of each sex. Repeated dose oral study was conducted as a preliminary to a long term study preferably animals from the same strain and source were used in both studies.

Housing and feeding conditions

The temperature in the experimental animal room was maintained at 22^0C ($\pm 3^0$C). The relative humidity was 50-60% (not exceed 70%) and the lighting sequence was 12 hrs light and 12 hrs dark. For feeding, conventional laboratory diet was used with an unlimited supply of drinking water. Animals were housed in small groups of same sex (NMT 5 animals in each cage)

Preparation of animals

Healthy young adult animals were randomly assigned to control and treatment groups. Cages were arranged in such a way that possible effects due to cage placement were minimized. The animals were identified uniquely and kept in their cages for five days prior to the start of the study to allow for acclimatization to the laboratory conditions.

Table 6-Dose of drug for Sub acute Toxicity Studies

Group	Treatment
I	Control
II	Therapeutic dose
III	Medium dose
IV	High dose

Dose administration

The leaf extracts were administered by oral gavages. Ten animals (5 males and 5 females) were used at each dose level for each extracts. Three test groups and a control group were used for both extracts and the highest dose level was chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter a descending sequence of dose levels selected with a view to demonstrating dosage related response and non-observed-adverse effects at the lowest dose level (NOAEL). The animals were dosed with test substance daily 7 days each week for a period of 28 days.

FUNCTIONAL OBSERVATIONAL BATTERY (FOB) OBSERVATIONAL PROCEDURES

Home-cage observation

The animals were observed closely from outside the cages without agitating them; body position, respiration, clonic involuntary movement, tonic involuntary movement, vocalizations and palpebral closures were noted[41].

Hand-held observations

The reactions of the animals were observed when they were removed from their cages and held. Reactivity, palpebral closure, lacrimation, salivation and pilo erection were noted. Detail any finding related to dirtiness of hair cast, bite marks,

missing nails, gauntness (stomach could be touched, median vertebrae protrude) or death (findings) [42].

Open field activity

Each animal was gently placed in the centre of a field and made the following 10 observations for 3 minutes [43]

a) Rearings

b) clonic involuntary movement

c) Tonic involuntary movement

d) Gait d) Movements

e) Arousal f) Stereotype

g) Abnormal behavior

h) Defecations

i) Urinations.

Stimulus response

Approach response; touch response, eyelid reflex, pinna reflex, sound response, tail pinch response, tail flick latency, pupillary reflex, righting reflex observations were carried out:

Eyelid reflex assessment

The animal's eyelid was touched with a dull object (cotton buds) and blinking response was noted.

Pinna reflex assessment

This is to assess the sensor motor activity of the animals. The fine object (cotton) was slightly touched inside the ear of the animals. The time duration taken for the response was noted.

Sound response assessment

In order to assess the sensor motor activity of the animals, the fingers snapped above and behind the animals head. The time duration taken for the flinch and flicks were scored.

Pupillary response assessment

The pen light was shined in the eyes of the animals. The response to contraction was scored.

Response to visual stimuli

The blunt object was approached the animals head and held the object 1 to 2 inch from the animal's head for few seconds. The time duration taken for approaching the object was recorded.

Tail flick latency assessment

This method was used to assess the sensor motor status of the animals[40] Response to painful stimuli was measured by the tail flick method using anlgesiometer. In this the animals were exposed to noxious stimulus like radiant heat and tail flick latencies (the time required for the flicking of tail i.e. the reaction time and a mean of two pre drug recordings were taken as basal value(0minute). A cut off time of 10 seconds was maintained in order to prevent tissue injury (based on the reaction time that generally varied between 3-4 seconds).

Tail pinch response

This was used to assess the sensor motor activity of the animals and the response to painful stimuli was measured in this method. The animal's tails were lightly pinched approximately 2 inch from the tip with the help of forceps. Recordings were noted.

NERVOUS AND MUSCLE MEASUREMENTS

Motor coordination assessment

For the evaluation of coordination and balance, the rotarod test was carried out. The apparatus consists of a horizontal metal rod positioned 25cm above a switch floor. The rod was divided in to four parts by plastic plates so that four rats could be tested at a time. Rats were positioned on the rod rotating at a constant speed of 20 rpm and the time the rat could stay on the rod without falling was recorded[44].

Locomotor activity assessment

Locomotor activity is determined by using actophotometer and this is to assess the neuromuscular status of the animals. The animals were placed on the digital actophotometer. The locomotion of the animals was recorded.

Righting reflex assessment

This is also used to assess the neuromuscular status of the animals. In this the animals are placed on their back. The ability to regain a position on all four legs was recorded.

Landing foot splay assessment

This method was used to assess the neuromuscular status of the animals. The animals were grasped by the scruff of the neck and base of the tail. The paws were marked with ink and held above the bottom at a height of 1 ft. The animals were released and the distance between the heels was measured. The foot play distance was measured.

Body weight

The body weights were recorded at the study Day 0, weekly during the study and at scheduled necropsy.

Feed and water consumption

Food consumption was assessed on a daily basis by weighing the feeders and expressed as grams per rat per day. Water consumption was measured.

Hematology and biochemistry

A complete battery of haematological, clinical chemistry, and urinalysis measurements were taken at baseline and during the final week of the study on the animals per sex from each dose group. The selected animals were placed in metabolic cages, during which time urine was collected for urine analysis. Blood was also collected from the animals for fasting glucose analysis.

Haematological parameters evaluated included haemoglobin, packed cell volume (PCV); erythrocyte count, leukocyte count (total and differential), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated and reported. Clinical chemistry parameters evaluated on separated serum samples included alkaline phosphates (ALP), SGOT, SGPT, Albumin, Protein, glucose, urea, creatinine, serum TSH level.

All surviving test and control animals were sacrificed at study termination by cervical dislocation under anaesthesia and subjected to a complete necropsy. The organs were harvested and weighed and relative organ weights (g/kg body weight) were calculated using terminal body weights.

Gross pathology

All animals in the study were subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys,

adrenals, testes, epididymis, prostate with seminal vesicle with coagulating glands as a whole, thymus, spleen, brain and heart of all animals (apart from those found moribund and/or intercurrently killed) trimmed of any adherent tissue, as appropriate, and their wet weight taken.

The following tissues were preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain, liver, kidneys, adrenals, spleen, heart, thymus, trachea and lungs (preserved by inflation with fixative and then immersion), epididymis, prostate + seminal vesicle, lymph and peripheral nerve.

Statistical methods

Body weight data were analyzed using a one-way analysis of variance followed by Dennett's multiple comparison tests $P<0.05$ was considered to be statistically significant. One-way analysis of variance (ANOVA) was used in analyzing body weight change, organ weight, and food and water consumption.

PHARMACOLOGICAL SCREENING

Male Swiss albino rats weighing 150-200g were used for the present work. The animals used for the experiment were maintained under standard laboratory conditions in an animal house of RVS College of Pharmaceutical sciences approved by the committee for the purpose of control and supervision on experiments on animals (Ref.No: IAE1012/C/06/CPCSEA) under 12 h dark/light cycle and controlled temperature 24 ± 2^0C. They had free access to food and water *ad libitum.* The animals were acclimatized to the laboratory for a period of 7 days, before the commencement of experiment.

Oral glucose tolerance test[44,45]

The ability of an individual rat to handle a standard oral glucose load was evaluated by assessing the blood plasma for glucose level. Study on Oral Glucose Tolerance Test (OGTT) initially, hypoglycemic activity of herbal formulation was carried out in overnight fasted normal rats, which were equally divided into four groups of six rats each. Normal control group received only vehicle (1 ml of water) and test group received the formulation in three different dose level ie low, therapeutic and high dose respectively. Following 30 min post extract administration all the animals were fed with glucose (2 g/kg). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration.

The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diabnostics, GmbH, Germany).

ANTIDIABETIC ACTIVITY

INDUCTION OF DIABETICS[46-48]

Six adult albino rats weighting 250-300 grams (75-90 days old) were used for inducing diabetes. The animals were injected by Streptozotocin at the dose of **55 mg/kg** of the body weight intravenously. Streptozotocin induces diabetes within 3 days by destroying the beta cells. Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control. Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones. Food consumption was measured in terms of (gr.), water consumption was measured in terms of (ml) and urine volume was measured in terms of (ml) on a daily basis while every 2-4 weeks in 80 days the levels of C-peptide, insulin and glucose in blood serum were also measured, so that chemical diabetes was verified in rats injected with Streptozotocin

ASSESSMENT OF DIABETIC.

Diabetic was conformed after 48 hr of streptozotocin injection, the blood samples were collected through retro orbital puncture and plasma glucose level were estimated by enzymatic GOD POD diagnostic kit method. The rat having fasting plasma glucose levels more than 250 mg/dL were selected and used for this study.

EVALUATION OF EXTRACT ON STREPTOZOTOCIN INDUCED DIABETIC RATS.

The albino rats on either sex have been selected for the experimental study. The weight of the should be around 170-240 gm. The animals are divided into six groups. Each group has 6 animals.

Group 1was kept as normal (normal rat) received only distilled water; group 2 was kept as negative control, Streptozotocin induced and received only water. Group 3 was treated with glibenclamide (10mg/kg) Group 4, 5 and 6 is diabetic induced rat and treated with 100mg/kg,200mg/kg and 400mg/kg b.w of ethanol extract of *Polialthia longifolia.*(EPLR)

Table 7 - Evaluation Of Extract On Streptozotocin Induced Diabetic Rats

S no	Groups	Treatment	
1	Group I	Normal control	
2	Group II	Diabetic control	
3	Group III	Diabetic + glibenclamide(5 mg/kg)	
4	Group IV	Diabetic + Extract Lower dose	
5	Group V	Diabetic + Extract Middle Dose	
6	Group VI	Diabetic + Extract Higher Dose	

EPLR *was* administered for 21 days at a three different dose levels 100, 200 and 400mg/kg. Dried extract made in aqueous and given orally. The blood was collected by sinus orbital under the light diethyl ether anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Body weight glucose was analyzed every week and lipid and lipoprotein profile from serum (TC, TG, HDL, LDL, VLDL.) were analyzed after 28 days.

OBSERVATION

Serum glucose level estimation (initial and final)

Body weight of the albino rats (initial and final)

ESTIMATION OF LIPIDS.

TOTAL CHOLESTEROL[49]

The cholesterol in serum was estimated by using ecoline diagnostic kit. Cholesterol and its ester were released from lipoprotein by detergents cholesterol estrates hydrolyses the ester the sub subsequent enzymatic oxidation by cholesterol oxidize, Hydrogen peroxidase was formed. This was converted into colored quininemine in a reaction with 4- aminopyantipyrine and phenol catalyses by peroxidase the observance of the sample and of the standard was measured against the reagent blank value at 546nm. Cholesterol level in serum was expressed as mg/dL.

TRIGLYCERIDES.

The triglyceride level was estimated by using Ecoline diagnostic kit.

Triglyceride $\xrightarrow{\text{Lipase}}$ Glycerol + Fatty acid

Triglyceride $\xrightarrow{\text{GK}}$ Glycerol + Fatty acid

Glycerol 3 phosphate + O2 $\xrightarrow{\text{GPO}}$ Dihydroxy acetone phosphate+ H2O2

2H2O2 + Aminoantipyrine + 4 chlorophenol $\xrightarrow{\text{POD}}$ Chinonimine + 4H2O

The absorbance of the sample and of the standard was measured against reagent blank value at 546nm. Triglyceride level in serum was expressed as mg/dL.

HDL CHOLESTEROL

The cholesterol was separated from the serum after precipitation of LDL cholesterol by phosphotungstic acid precipitating reagent. The supernatant. After centrifugation was estimated using Ecoline diagnostic kit. The absorbance of sample and of the standard was measures against the reagent blank value at 546 nm. HDL cholesterol level in serum was expressed as mg/dL.

LDL CHOLESTEROL.

The LDL Cholesterol is calculating by following formula

LDL Cholesterol = Total cholesterol – [HDL cholesterol – Triglyceride / 5].

LDL cholesterol level in the plasma is calculated and expressed in the unit of mg/dL.

VLDL CHOLESTEROL.

The VLDL Cholesterol is calculating by following formula

VLDL Cholesterol = Total cholesterol – HDL cholesterol – Triglyceride-LDL.

6. RESULTS

Table 8 - Soxhlet Extraction Of Polialathia Longifolia Roots

Plant	Part used	Method of Extraction	Solvents	Weight of Powder taken (A)	Wight of product (B)	Percentage Yield (W/V)
Polialthia longifolia	ROOT	Continuous Hot percolation by Soxhlet apparatus	Ethanol (60-80°C)	50	2.88	5.96

A = Weight of powder plant material

B = Weight of extract

Percentage yield = **(B/A) X 100**

Preliminary phyto chemical screening.

EEPLR extracts was subjected various chemical tested as per the standard methods for the identification of the various constituents. The result if this phyto chemical analysis is listed below.

Table-9 Qualitative phyto chemical screening of EPLR

Plant constituent	Ethanolic Extract
Steroids	**-**
Carbohydrate	**+**
Flavonoids	**+**
Proteins and amino acids	**-**
Glycosides	**-**
Alkaloids	**-**
Saponins	**+**
Volatile oil	**+**
Tannins	**-**

"+" Presence, "-" Absence.

TOXICITY STUDIES

ACUTE TOXICITY STUDIES

Acute toxicity studies on the albino rats show no morality at a dose of 2000mg/kg, during a time period of 14 days. During the study, no noticeable were seen in the rats. This help to predict that it does not contain any type of toxicity and it is full safe. So 200 mg/kg b.w ($1/10^{th}$ and 500mg/kg b.w ($1/4^{th}$) and 1000mg/kg ($1/2^{th}$) were selected of that dose for the further study.

SUBACUTE TOXICITY STUDIES

Functional observations are necessary to test the neurotoxic effects in the assessment of the toxic effects of ICFE. The functional observational battery is a non-invasive procedure designed to detect gross functional deficits in animals resulting from exposure to chemicals and to better quantify neurotoxic effects detected in other studies. This battery of test is not intended to provide a detailed evaluation of neurotoxicity. It is designed to be used in conjunction with neuropathologic evaluation and/or general toxicity testing. Additional functional is necessary to assess completely the neurotoxic potential of drug.

Table 10- Effect of EPLR on Stimulus Response

Parameters	Observations
Approach response	Slowly approaches, sniffs and pulls back/normal
Touch response	Slowly retreats/normal
Eyelid response	Blinks/normal
Pinna reflex	Auricle twitches/normal
Sound response	Mild reaction, hears sound/normal
Tail flick response	Flicks the tail or normal
Pupillary reflex	Contracts
Righting reflex	Lands on four limbs/normal

Table-11: Effect of EPLR on Autonomic Observations

S L N O	**Parameters**	**Observations**
1	Reactivity	Easy/normal
2	Handling	Did not resist, very easy to handle
3	Palpebral closure	Normal (eyes are open)
4	Lacrimation	No lacrimation
5	Salivation	No salivation
6	Piloerection	None/normal
7	Hair coat	Normal
8	Bite marks	None
9	Nail status	Normal
10	Rearing activity	Normal
11	Clonic involuntary movement	None
12	Tonic involuntary movement	None
13	Gait	Normal
14	Movements	Normal
15	Arousal	Normal (keeps guard up and engages in exploratory activity)
16	Stereotype behaviour (preening, squeaking, shaking head and other repetitive behavior	None
17	Abnormal behaviour (squirming, running backwards, labored movements, squealing)	None

Table 12- Effect of EPLR on Nervous and Muscle Measurements

Parameters	Observations
Abdominal tone	Normal (proper hardness)
Limb tone	Normal
Motor coordination	No abnormal changes
Landing foot splay	No significant difference between control

Table 13 Effect of EPLR on Motor Coordination Assessment

Group	Control	Low dose	Medium dose	High dose
Male	10.21±0.66	12.58±0.93	11.65±0.84	12.03±0.82
Female	10.8±0.52	12.3±0.98	11.74±0.78	10.78±0.65

Values are expressed as mean±SEM (N=5) and not significant statistically.

Figure 8: Effect of EPLLR on Motor coordination Assessment

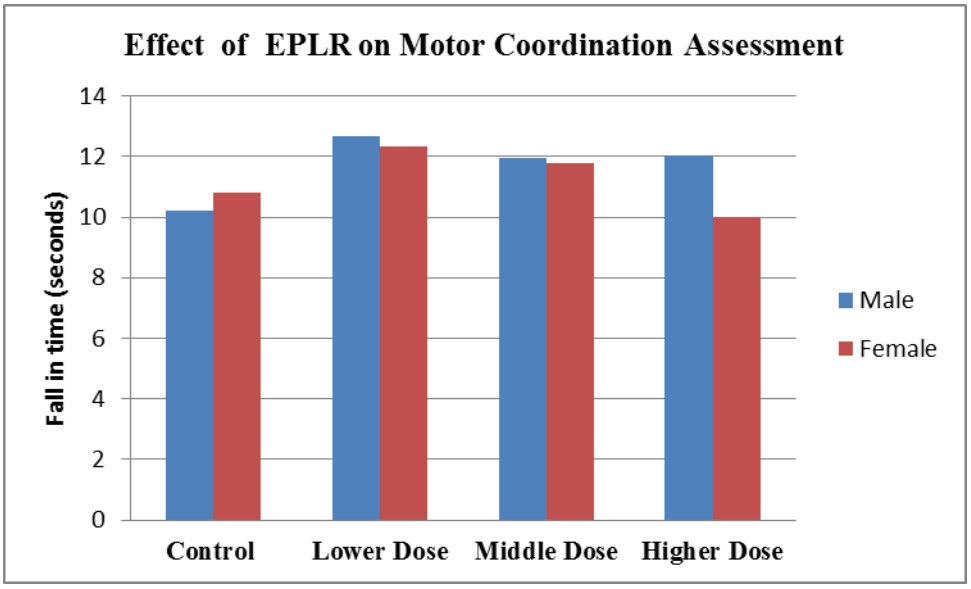


Table 14- Effect of EPLR on Locomotors Activity Assessment

Group	Control	Lower dose	Middle Dose	Higher Dose
Male	141.3±2.02	145.4±4.15	141.±2.77	138.2±3.16
Female	153.6±2.65	122.2±3.10*	126.6±3.37	124.2±2.58

Values expressed as Mean±SEM, number of animals in each group=6; * P <0.05

Figure 9: Effect of EPLR on locomotors activity

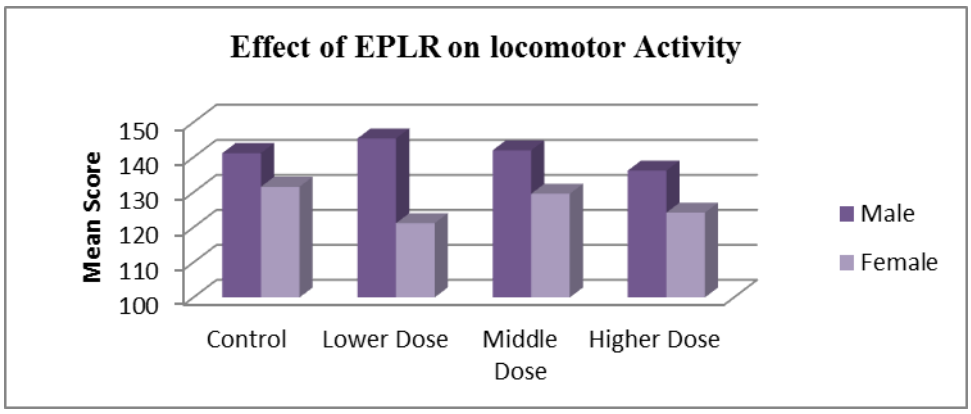


Table 15-Effect EPLR on Righting reflex Assessment

Group	Control	Lower Dose	Middle Dose	Higher Dose
Male	1.39±0.28	1.48±0.44	1.38.±0.432	1.44±0.248
Female	1.68±0.44	1.53±0.26	1.34±0.25	1.42±0.25

Values are expressed as Mean±SEM;

Number of animals in each group=5, not significant statistically.

Figure 10: Effect of EPLR on righting reflex

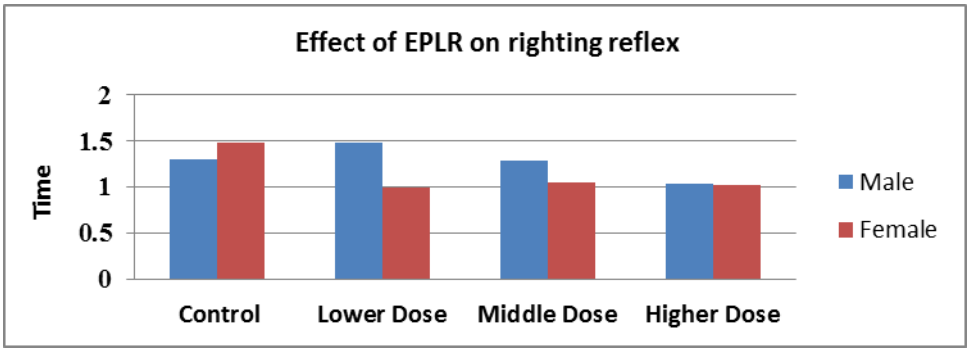


Table 16- Effect of EPLR on Tail Flick Latency

Group	Control	Lower Dose	Middle Dose	Higher Dose
Male	0.93±0.30	1.04±0.27	1.06±0.41	1.03±0.21
Female	0.92±0.03	1.08±0.22	1.09±0.26	1.01±0.23

Value are expressed as Mean ± SEM

Number of animals in each group=5, not significant statistically

Figure 11: Effect of EPLR on Tail Flick Latency

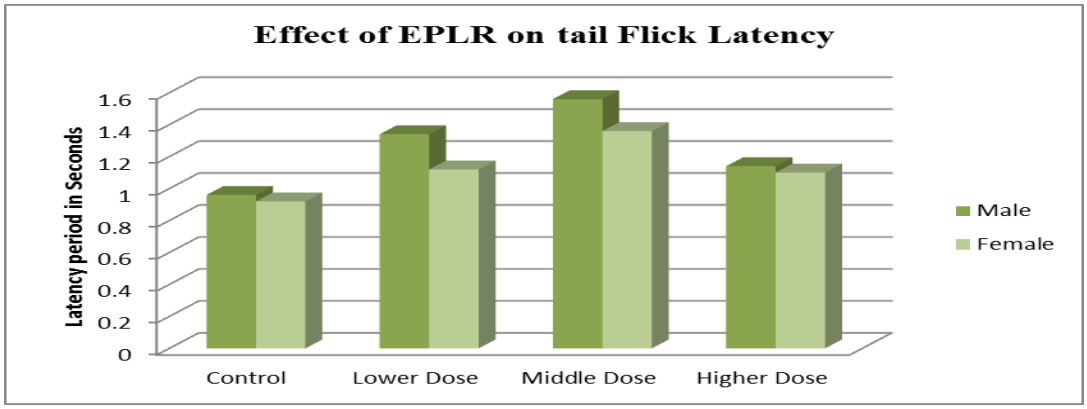


Table 17- Effect of EPLR on Pinna Reflex Assessment

Group	Control	Lower Dose	Middle Dose	Higher Dose
Male	2.10±0.20	2.08±0.37	2.06±0.41	2.10±0.32
Female	2.03±0.03	2.00±0.22	2.04±0.47	2.05±0.43

Values are expressed as Mean±SEM.

Number of animals in each group=5; Values- NS

Figure 12: Effect of EPLR on pinna reflex assessment

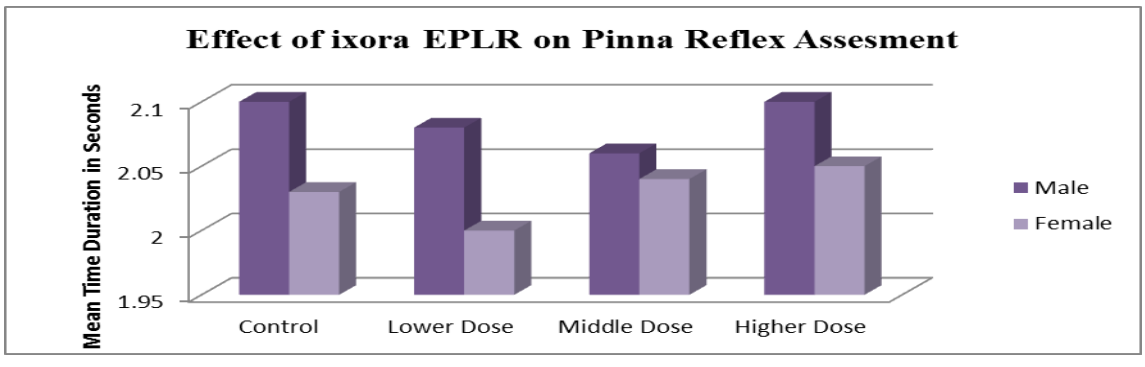


Table 18 - Effect of EPLR on Sound Response Assessment

Group	Control	Lower Dose	Middle Dose	Higher dose
Male	0.7±0.10	0.7±0.17	0.6±0.31	0.7±0.23
Female	0.6±0.06	0.5±0.20	0.6±0.45	0.6±0.42

Values are expressed as Mean±SEM.

Number of animals in each group=5; Values- NS

Figure 13: Effect on EPLR on Sound Response Assessment

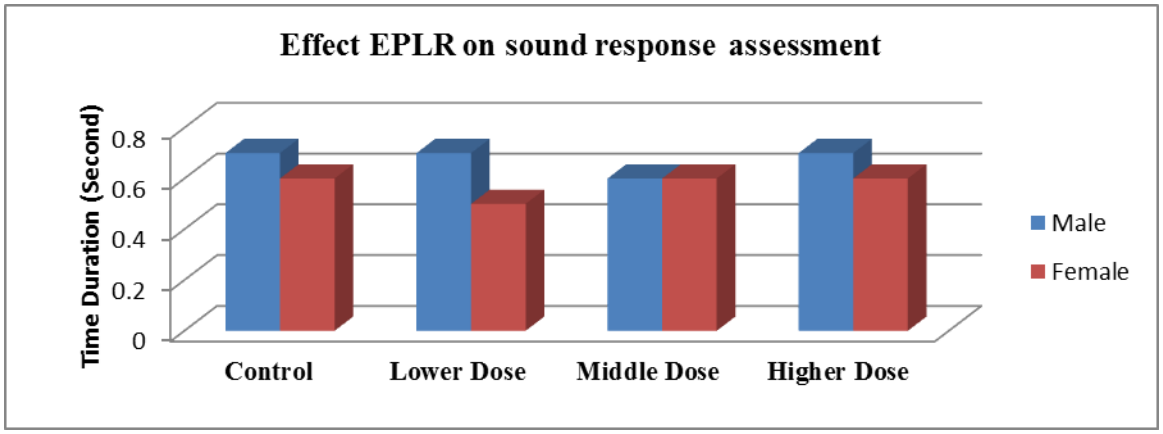


Table 19-Response To EPLR On Visual Stimuli

Group	Control	Lower dose	Middle dose	Higher Dose
Male	1.8±0.20	1.7±0.18	1.6±0.32	1.7±0.23
Female	1.6±0.42	1.5±0.24	1.6±0.47	1.7±0.32

Values are expressed as Mean±SEM;

Number of animals in each group=5; Values-NS

Figure 14: Effect on EPLR on visual stimuli

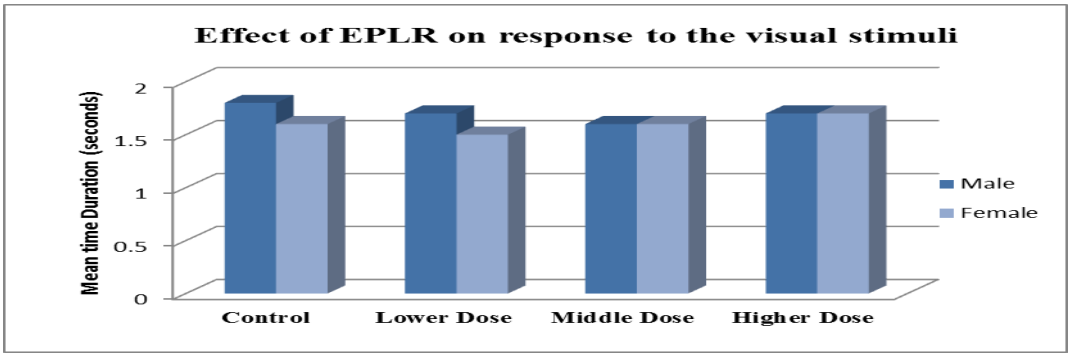


Table 20- Effect of EPLR on Percentage Change In Body Weight

Sex	Dose	Days					% change in body wt
		0	7	14	21	28	
Male	Control	129.4±3.26	139.8±2.37	148.2±2.33	158.8±1.85	169.8±3.15	31.22±0.9
	Lower Dose	137.2±2.82	144.4±1.69	154.6±1.63	164.±2.07	173.8±2.70	26.6±2.4
	Middle dose	131.2±2.35	135.4±4.31	139.09±0.89	145.5.±1.51	146.8±1.59	0.4±2.5
	Higher Dose	135.4±4.0	133.±2.55	138.8±2.53	143.4.±2.42	148.1±1.70	15.3±1.4
Female	Control	123.9±3.20	130±3.92	139.4±3.76	150±5.15	160.2±4.32	29.6±3.81
	Lower Dose	125.2±2.92	129±2.43	127.1±4.2	134.1±4.16	140.8±3.26	15.06±3.2
	Middle dose	122.4±2.27	133±2.40	132.8±2.06	138.2±2.18	142.2±1.24	20.2±1.3
	Higher Dose	123.2±2.65	125.6±2.18	128.8±1.28	134.6±1.20	141.6±1.99	18.4±4.2

Figure 15: effect of EPLR on body weight

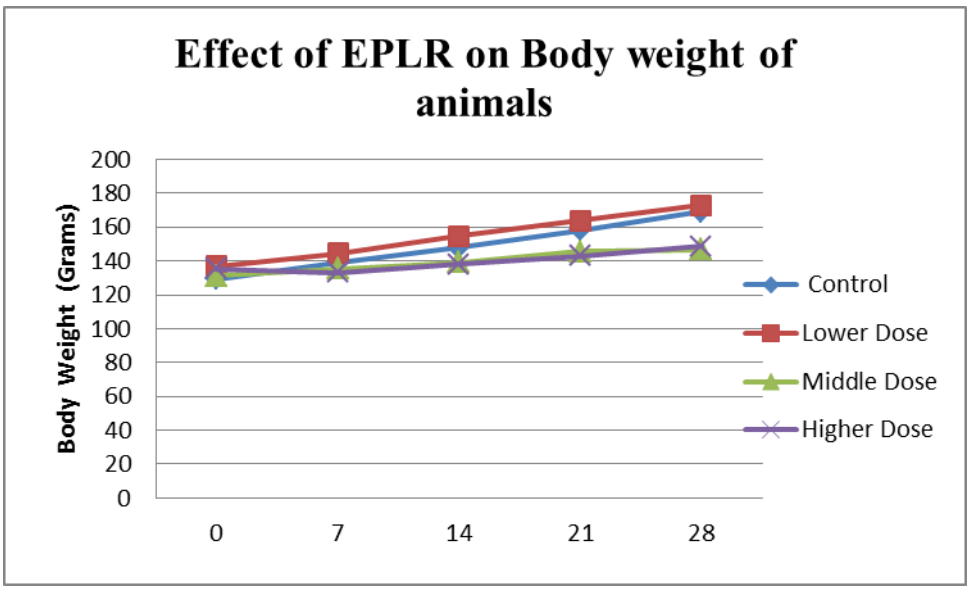


Table 21 –Effect of EPLR on Relative Organ Weight

Sex	Group	Brain	Heart	Liver	Kidney
Male	Control	1.35±0.12	5.18±.12	5.63±3.4	5.73±.43
	Low dose	1.37±.13	5.20±1.3	5.731.2±1.22	5.82±.26
	Medium dose	1.34±1.5	5.25±1.3	5.80±3.2	5.90±3.5
	High dose	1.40±2.3	5.28±2.3	5.85±1.2	5.99±4.2
Female	Control	1.50±1.56	5.82±.34	5.83±.45	6.12±1.3
	Low dose	1.45±2.34	5.94±1.76	5.92±2.43	6.23±.23
	Medium dose	1.40±3.53	6.15±.54	6.12±.4	5.98±2.7
	High dose	1.42±1.43	6.27±.32	5.85±6.3	6.09±6.4

Figure 16: Effect of EPLR on organ weights

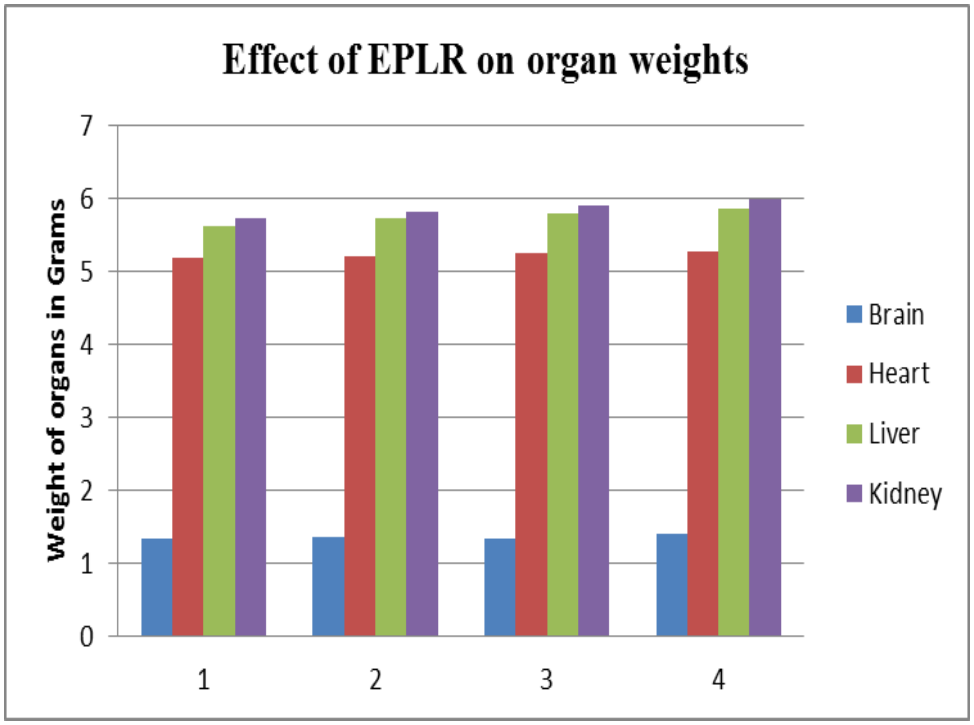


Table 22-Effect of EPLR on serum biochemical parameter

Sex	Group	Days	Hb g/dl	RBC $\times 10^6 mm^3$	WBC ($\times 10^3$ Mm^3)	Differential WBC count				
						N %	L %	E %	M %	B %
Male	Control	0	7.8±2.34	2.67±0.67	7.07±0.31	32.56±7.89	65.8±7.15	0.93±1.3	0	0
		28	7.9±1.00	2.72±0.32	6.87±.62	32.66±7.09	65.66±7.63	0.67±0.58	0	0
	Lower Dose	0	7.8±2.3	2.56±0.65	7.1±.88	29.93±4.54	67.8±7.23	1.20±1.48	0	0
		28	7.7±2.02	2.42±0.60	7.3±.63	30.33±4.50	68.66±3.51	1.21±1.00	0	0
	Middle Dose	0	7.8±2.3	2.67±0.88	7.06±.31	29.01±12.54	71±2.72	0.67±2.88	0	0
		28	8.2±1.25	2.72±0.45	6.86±.62	28.33±10.5	72±10.00	0.67±0.58	0	0
	Higher dose	0	7.6±2.54	2.64±0.76	7.2±.31	32.66±4.43	66.43±11	1.22±1.30	0	0
		28	7.7±0.57	2.59±0.15	7.4±.32	33.35±9.07	65.33±8.50	1.30±0.58	0	0
Female	Control	0	7.9±2.34	2.67±0.67	6.50±2.2	32.56±7.89	64.8±7.15	0.92±1.3	1.4±0.64	0.4±0.78
		28	7.9±1.00	2.72±0.32	7.00±1.57	31.66±7.09	65.66±7.63	0.67±0.58	1.5±0.57	0.6±0.69
	Lower Dose	0	7.7±2.3	2.56±0.65	5.9±2.2	30.93±4.54	66.8±7.23	1.33±1.48	1±1.4	0.4±0.54
		28	7.7±2.02	2.42±0.60	6.01±1.52	30.33±4.50	66.66±3.51	1.26±1.00	0	0.8±0.83
	Middle Dose	0	7.8±2.3	2.67±0.88	7.084±2.99	29.01±12.54	72±2.72	0.68±2.88	1±1	0.5±0.8
		28	8.2±1.25	2.72±0.45	6.56±8.6	28.33±10.5	74±10.00	0.69±0.58	0.4±0.5	1.0±1.0
	Higher Dose	0	7.6±2.54	2.64±0.76	6.65±1.38	33.66±4.43	67.43±11	1.20±1.30	0.9±0.83	0.2±0.44
		28	7.7±0.57	2.59±0.15	6.85±4.2	34.33±9.07	68.33±8.50	1.17±0.58	0	0.2±0.4

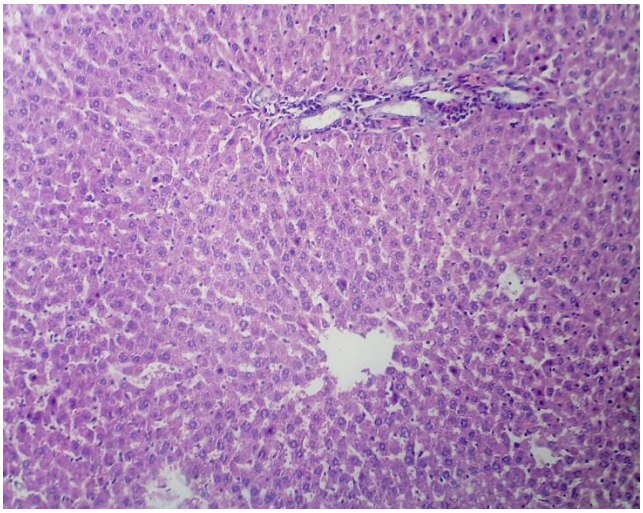
Values are expressed as Mean ± SEM; not significant statistically.

Table 23-Effect of EPLR on serum biochemical parameter

Sex	Group	Days	Serum creatinine mg/dl	Serum SGOT	Serum SGPT
MALE	Control	O day	1.3 ±0.34	22.22±3.42	30.09±1.21
		28th day	1.2 ±0.2	21.63±5.51	31.13±1.21
	Lower Dose	O day	1.36 ±0.31	29.56±5.34	29.99±5.64
		28th day	1.46 ±0.23	24.87±7.08	28.03±5.58
	Middle Dose	O day	1.40 ± 0.42	34.67±9.89	37.98±9.61
		28th day	1.83 ± 0.2	36.613±10.39	40.40±9.61
	Higher dose	O day	1.32 ± 0.2	29.97±12.42	34.0±15.32
		28th day	2.25±0.28	30.95±14.26	34.9±14.17
FEMALE	Control	O day	0.9 ±0.44	35.84±1.34	42.53±4.24
		28th day	0.89 ±0.4	36.2±5.03	40.23±1.83
	Lower Dose	O day	1.06 ±0.20	39.02±4.13	41.84±4.7
		28th day	1.18 ±0.13	40..04±2.2	40.84±4.3
	Middle Dose	O day	1.36 ± 0.42	40.05±6.4	46.34±5.56
		28th day	1.76 ± 0.2	38.95±5.4	47.32±4.32
	Higher Dose	O day	1.05 ± 0.21	36.02±2.1	39.35±3.3
		28th day	2.07±0.28	39.04±4.22	40.43±5.23

HISTOPATHOLGICAL ASSESSMENT OF EPLR Extracts

Figure 17 - T.S of rat liver showing normal cells in subacute toxicity study of EPLR



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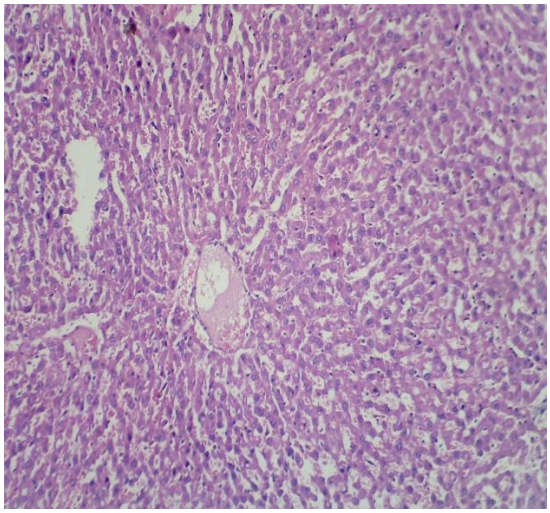
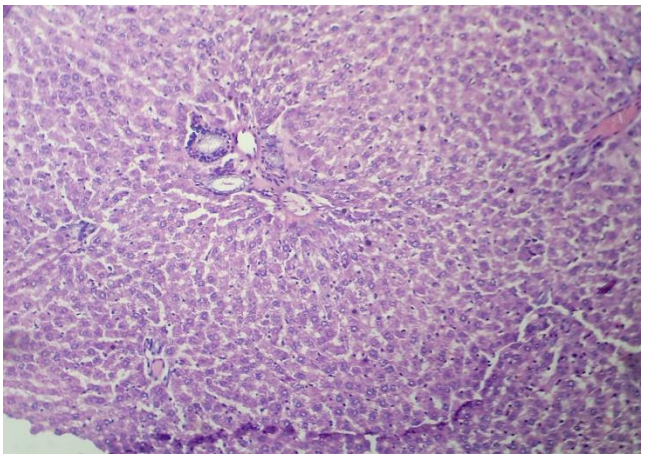
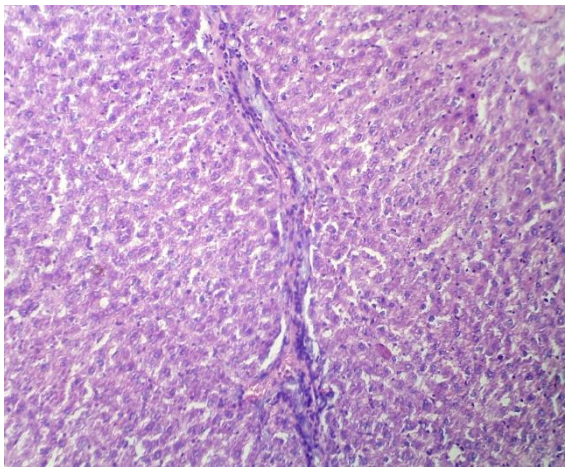


Lower dose



Medium dose



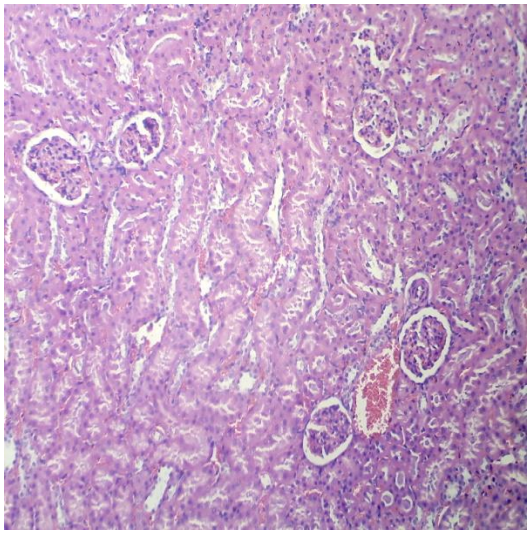
Higher dose

Microscopic appearance-

Section studies from the liver shows normal lobular architecture. Individual hepatocyte, central vein and sinusoids shows p- unremarkable. There is no evidence of inflammation and necrosis.

KIDNEY

Figure 18 - T.S of rat kidney showing normal cells in Sub Acute Toxicity Studies of EPLR



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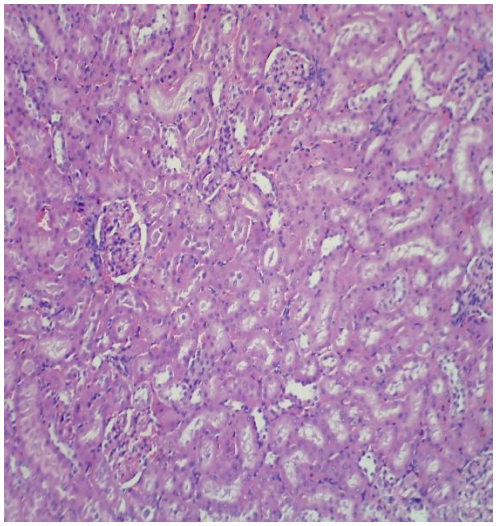

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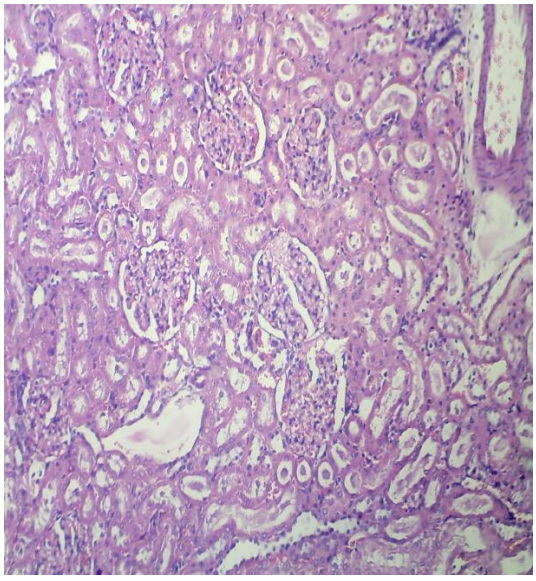

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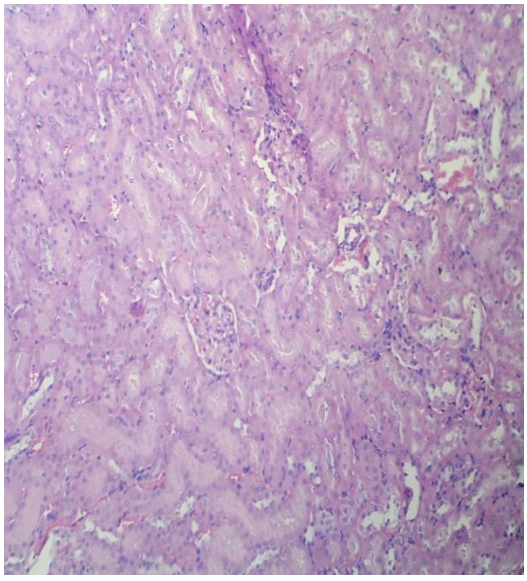


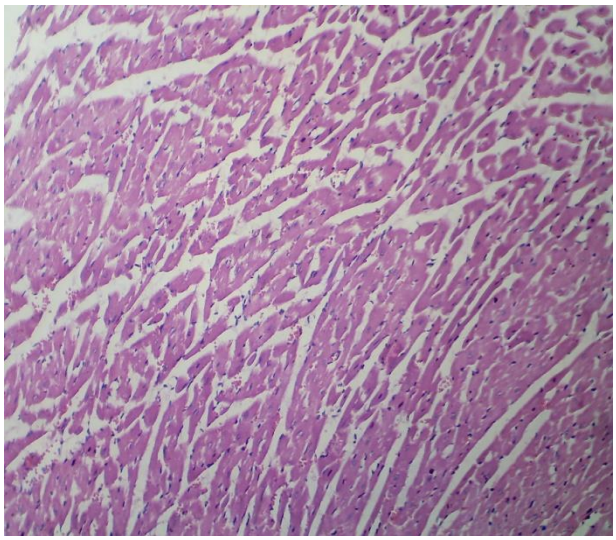
Higher dose

Microscopic appearance

Section studies from the kidney show normal cortex and medulla. The glomeruli, interstitium and blood vessels are unremarkable. There is no evidence of inflammation and necrosis.

HEART

Figure 19 - T.S of rat heart showing normal cells in Sub Acute Toxicity Studies of EPLR



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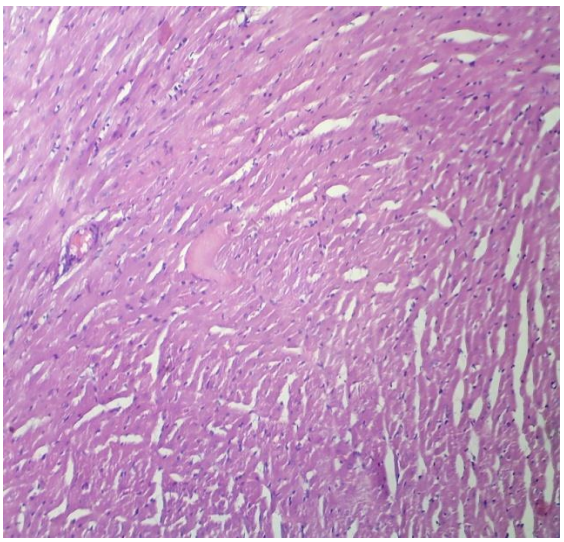
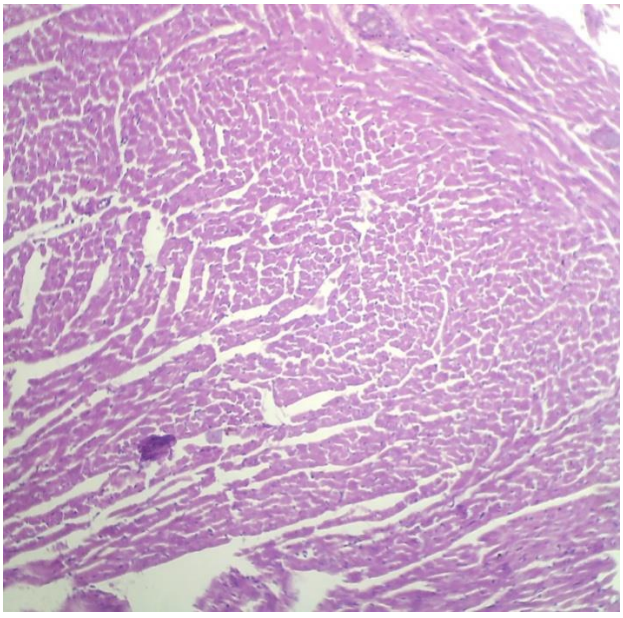


Lower dose



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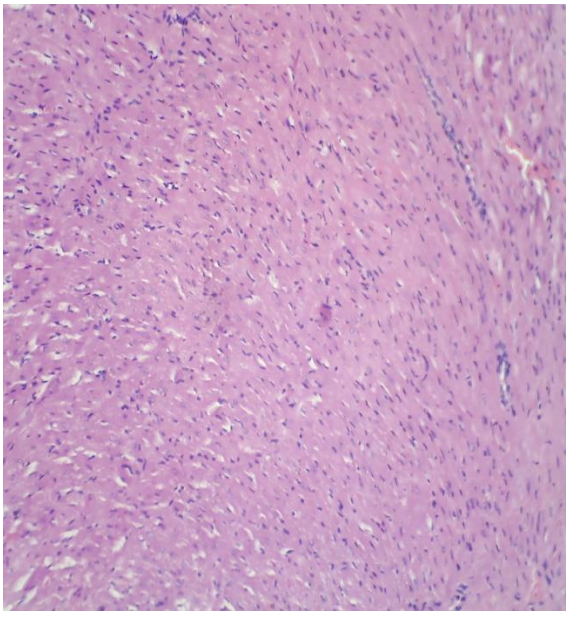


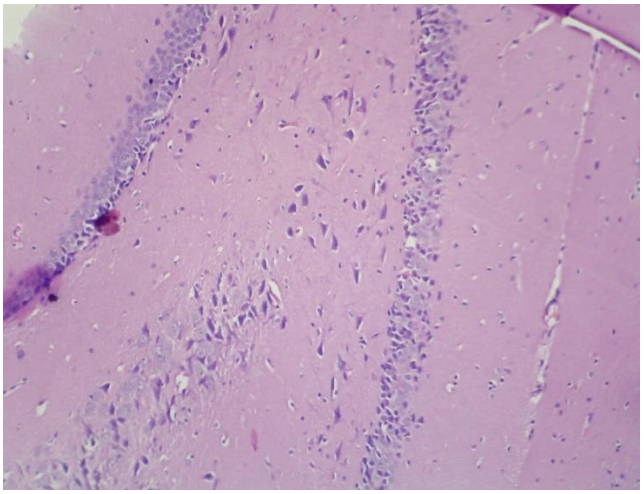
Higher dose

Microscopic appearance-

Section studies from the heart shows normal myocardium with myocytes. There is no evidence of myocytic degeneration or edema.

BRAIN

Figure 20 - T.S of rat heart showing normal cells In Sub Acute Toxicity Studies Of EPLR



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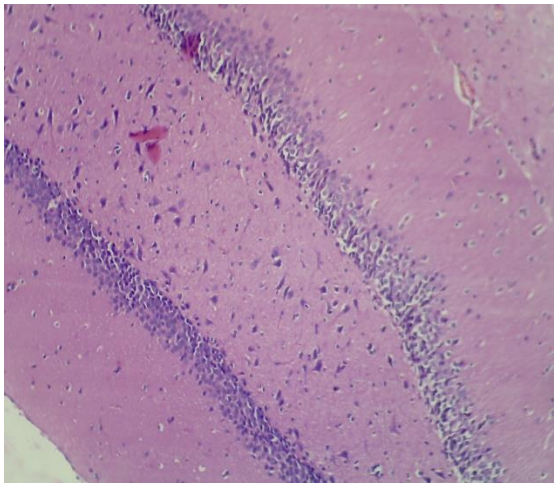
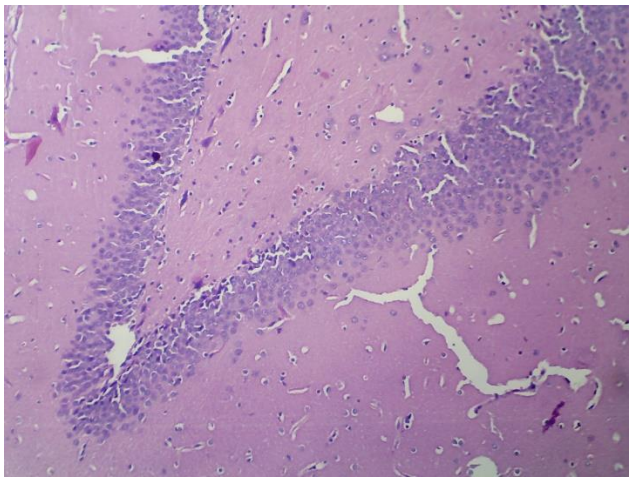


Lower dose



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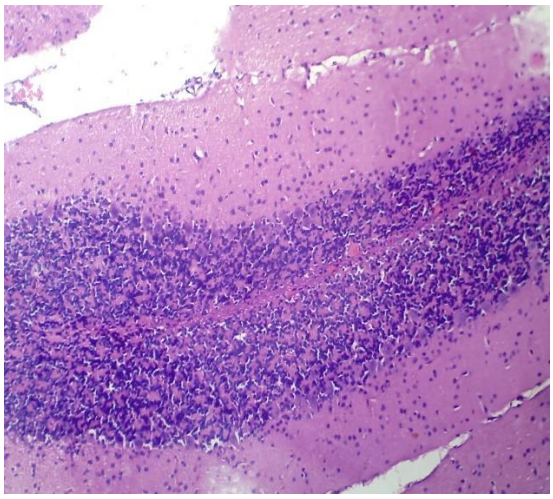


Higher dose

Microscopic appearance-

Section studies from the brain shows normal cerebellum. Brain parenchyma, purkinjic cells and basal ganglion unremarkable. There is no evidence of inflammation and necrosis.

ANTIDIABETIC ACTIVITY

Oral glucose tolerance test

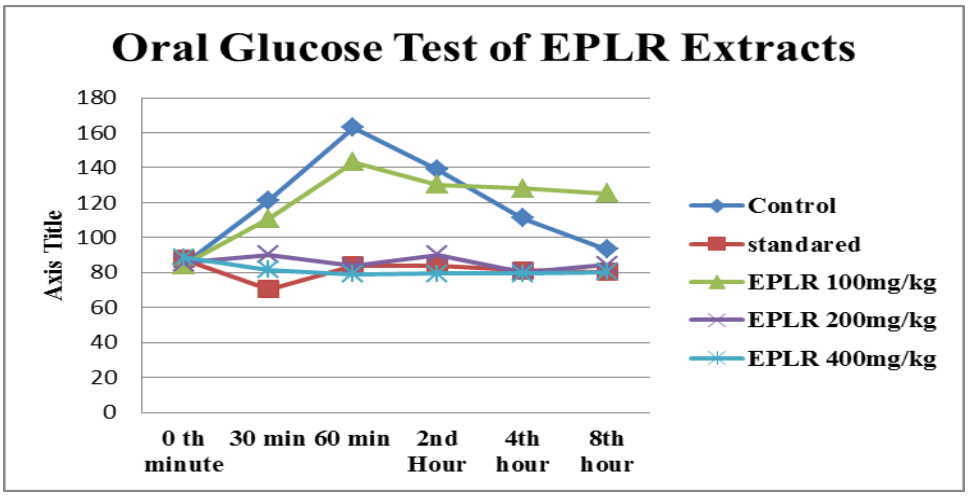
In the OGTT the EPLR extracts at a dose of 200mg/kg significantly reduced the blood glucose level at 30 minutes after glucose administration. Standard drug glibenclamide produced activity at all the time interval tested

TABLE 24- ORAL GLUCOSE TOLERANCE TEST OF *EPLR*

Group	Blood glucose levels (mg/dl)					
	0 min	**30 min**	**1st hour**	**2 nd hour**	**3rd hour**	**8th hour**
Control	**84.5 ± 1.14**	**121.4 ± 1.52**	**162.91 ± 13.67**	**139± 2.074**	**111.02 ± 5.805**	**93.4 ± 1.304**
Glibenglamide mg/kg	**87.5 ± 10.164**	**70.3 ± 7.19**	**84.0 ± 10.271**	**83.6 ± 13.342**	**81.2 ± 7.791**	**80.2 ± 7.328**
EPLR 100mg/kg	**84.3**	**110.8**	**143.12**	**130.12**	**128.12**	**125.22**
EPLR 200mg/kg	**85.6 ± 5.263***	**83.6 ± 6.894***	**83.6 ± 1.924***	**90.2± 2.408***	**80.4 ± 1.517***	**84.4 ± 2.302***
EPLR 400mg /kg	**88.5±1.22**	**81.5±0.22**	**79.05±1.22**	**79.3±2.5**	**79.5±2.5**	**80.3±2.1**

All value are expressed as mean ± SEM (n=6)

Figure 21 - ORAL GLUCOSE TOLERANCE TEST OF EPLR



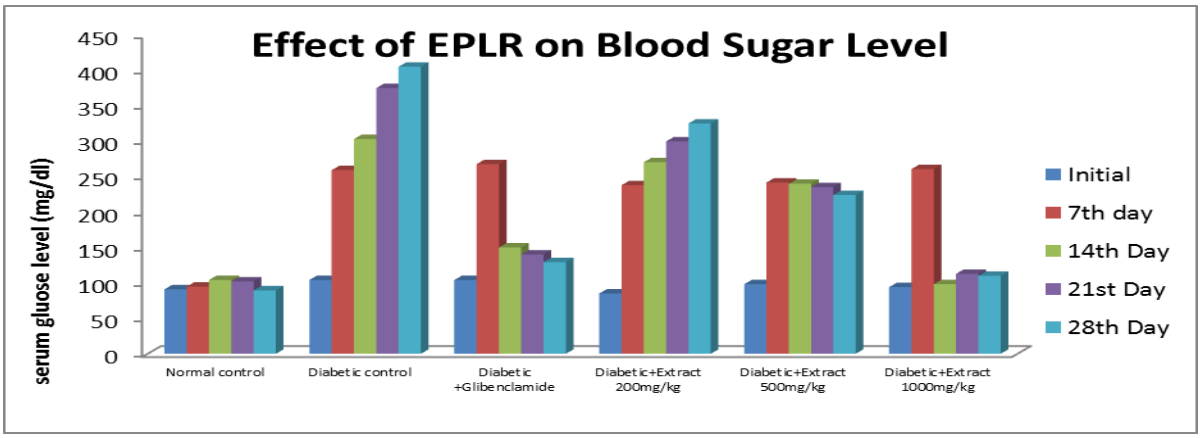
EFFECT OF *EPLR* ON SERUM GLUCOSE LEVEL

Table 25- -Effect of EPLR on serum glucose level in normal control and STZ induced diabetic rats

S.NO	TREATMENT	SERUM GLUCOSE LEVEL				
		Initial	7th day	14th day	21st day	28th day
1.	**Normal control**	90.97±1.47	94.97±1.47	104.3±4.1	102±2.13	89.52±2.16
2	**Diabetic control**	104.22	259.3±3.51	303.2±5.5	375.1±1.3	405.3±1.26
3.	**Diabetic + Standard**	104.12±1.2	267.8±3.15	150.1±1.4	140.2±0.5	129.5±1.0
4.	**Diabetic+ Extract 100mg/kg**	85.11±5.5	238.0±2.65	270.5±2.3	299.88±1.1	325.2±2.46
5.	**Diabetic+ Extract 200mg/kg**	98.2±1.22	241.9±1.25	240.1±0.7	235.1±1.7	224.2±2.49
6	**Diabetic+ Extract 400mg/kg**	94.1±5.5	260.8±7.8	98.3±1.5	113±1.3	110±3.89

All values are expressed in MEAN ± SEM (n=6)

Figure 22 : Effect of serum glucose level in normal control and STZ induced diabetic rat



EFFECT OF EPLR *ON* BODY WEIGHT

There was gradual increase in body weight in normal control while the diabetic control continues to lose the weight. However treated diabetic group gained 6.25%, 8.24% as compared with the diabetic control and diabetic treated towards normal range. Extract changes in the body weight shows in the tables.

Table 26 -Effect of EPLR on body weight in normal control and STZ induced diabetic rats.

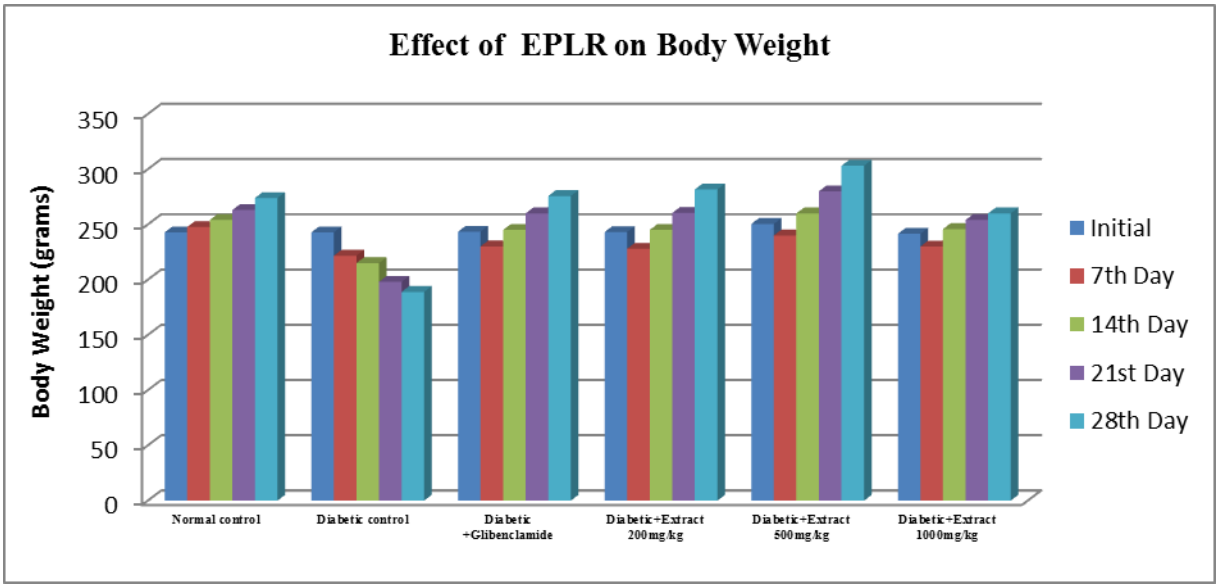
SL NO	TREATMENT	BODY WEIGHT				
		Initial	7th day	14th day	21st day	28th day
1.	Normal control	242.9±3.5	247.8±1.33	254.1±2.333	263.11±8.5	274±1.95
2.	Diabetic control	248.9±1.4	221.8±5.55	215.1±2.4	198.1±3.9	189±1.64
3.	Diabetic + Glibenclamide.	243.5±1.5	230.2±4.88	245.1±2.3	260.1±1.34	275.8±1.6
4.	Diabetic+ Extract 200	243.2±2.5	228.1±4.6	245.1±3.4	260.5±6.7	282.8±2.7
5.	Diabetic+ Extract 500	250.5±3.2	240.1±1.22	260.11±3.8	280.1±2.3	303.2±2.8
6.	Diabetic + Extract 1000	241.6±3.8	230.1±4.8	245.9±2.78	254.1±3.44	260.1±1.4

All value are expressed mean ±SEM (n=6)

P<0.001, as compared to diabetic control

P<0.001 as compared to Normal control.

Figure 23 : Effect on EPLR on Body weight



Effect of EPLR on Serum lipids

STZ diabetic rats group were found to have significantly increased VLDL, LDL, TG, TC level and markedly decreased HDL level as compared to normal control group. Treatment with EPLR extract 200mg/kg, 500 mg/kg and 1000mg/kg reduced significantly VLDL, LDL, TG, TC, levels and markedly increased HDL level as compared to diabetic control groups. Positive control was significantly preventing the increasing the serum TC, TG, LDL, VLDL and decreasing the HDL level as compared to diabetic group. Thus the extract treatment restored all these changes near to normal value. This change in serum is listed on the table.

Table 27--Effect Of EPLR On Serum Lipid And Lipoprotein Profile In Normal Control And STZ Induced Diabetic Rats

S.NO	Treatment	TC(mg/Dl)	TG(mg/Dl)	HDL(mg/Dl)	LDL(mg/Dl)	VLDL (mg/Dl)
1.	Normal control	87.16±4.5	85.62±1.6	42.5±1.32	91.4±1.07	46.5±1.32
2.	Diabetic control	155.8±7.4	189.86±4.	20.3±1.42	129±1.78	53.3±2.18
3.	Diabetic + Glibenclamide (10mg/kg)	92.3±2.35	101±2.90	45.4±1.48	102±2.56	33.2±1.65
4	Diabetic + extract (200mg/kg)	135.3±3.9	145.1±2.6	47.3±2.1	110.±2.62	41.3±1.56
5.	Diabetic + extract (500mg)	132±3.52	112±3.01	45.3±1.35	102±2.12	33.2±2.06
6.	Diabetic + extract (1000mg)	89.3±1.03	62.3±3.65	3.82±1.37	98.6±1.68	26.71±2.18

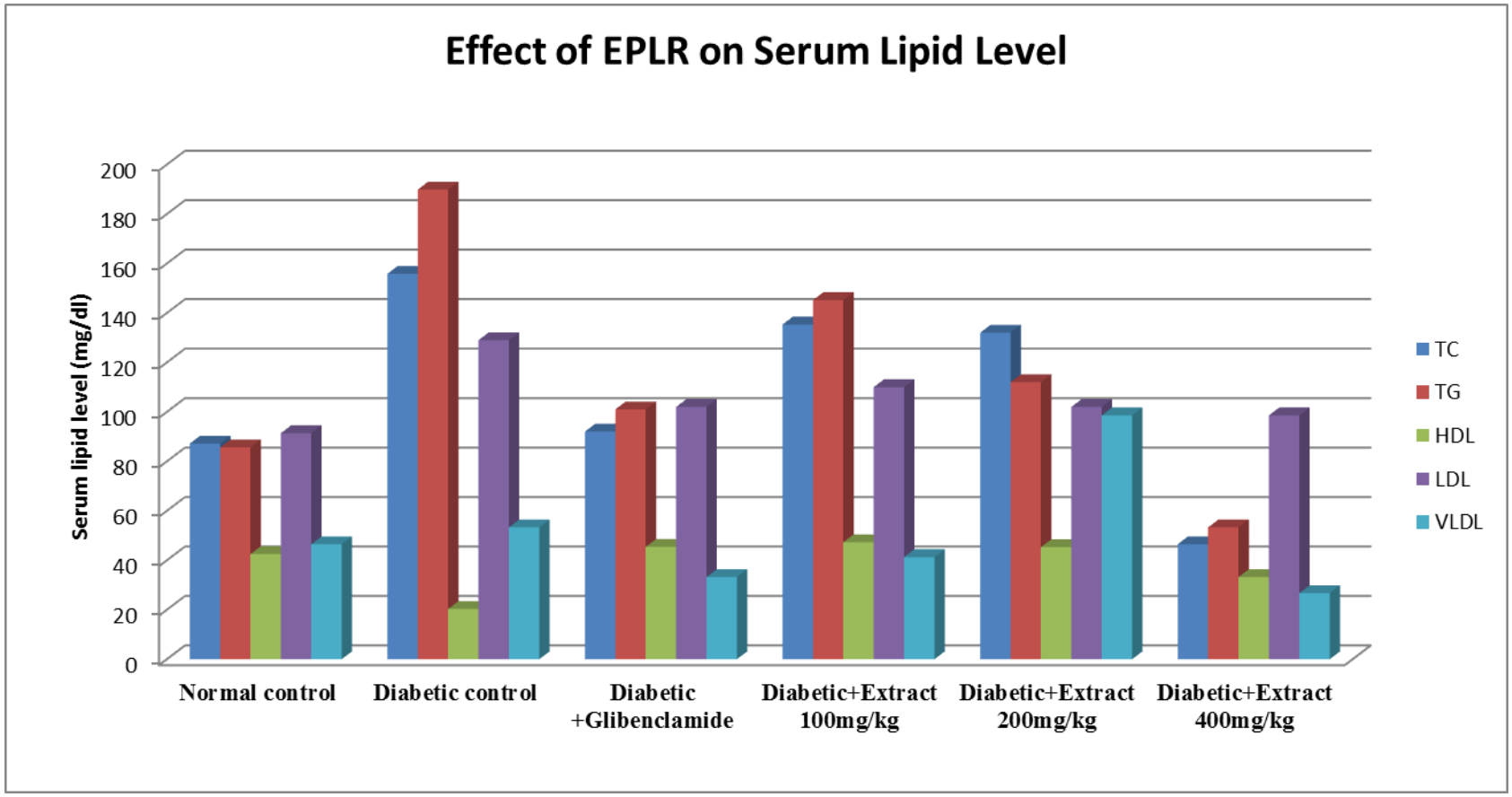
All value are expressed as mean ±SEM (n=6)

P<0.001, as compared to diabetic control

P<0.001 as compared to Normal control.

Table in parenthesis indicate % fall in body weight as compared to initial value.

Figure 24 : Effect of EPLR on Serum Lipid Level



Values are expressed as mean ± SEM of 6 animals in each group

7. DISCUSSION

Phytochemical screening

Qualitative Phytochemical screening and ethno botanical survey on the *Polyaltehia longifolia* root extracts the presented of certain phyto constitutions such alkaloids, tannins, carbohydrates, glycosides, terpenoids, high amount of volatile oils. The phytochemical constituents such as glycosides, tannins, triterpenoids, flavonoids, alkaloids may be linked to the anti diabetic activity.

Acute toxicity studies

To check the safety profile of the EPLR it was subjected to the acute toxicity study which conformed the absence of any toxicity or mortality at the higher dose of 2000mg/kg. Thus the EPLR can be classified as a safe drug category according to the Global harmonized Classification System quoted in the OECD guidelines 1996.

Based on the Toxicity studies 200mg/kg is used as a dose of extract and middle dose 500mg/kg(Dose×2.5) and higher dose as 1000mg/kg (Dose×5) used for sub-acute toxicity studies.

Sub-acute toxicity studies

Sub-acute toxicity study was done and the results shows nontoxic nature of both the EPLR. Also, all the animals from control and all the treated groups up to dose 1000 mg/kg survived throughout the dosing period of 28 days. Animals from all the treated groups exhibited comparable body weight gain with that of controls throughout the dosing period.. No significant changes in the organ weight were observed. Furthermore no specific cell damage was noticed by the microscopic examination. The central and autonomic profiles were normal throughout the study and no specific alterations were noticed.

The results of the effect of the extract on the body weight of the animals compared with vehicle are as shown in Table 24 and Fig. 17. There were no significant increases in the weight of animals treated with 200 mg ICFE. However, there were little amount of weight reduction body weight in middle dose and higher dose treated animals.

The results of the effect of EPLR on absolute organ weights of male and female rats are as shown in Table 21. Macroscopic examination did not show any changes in the colour of organs of the treated animals compared with vehicle. There were no significant changes in the relative weights of the liver, kidney and heart in both males and females. Treatment had no effect on spleen, stomach and testes of male rats.

Hematological analysis and Biochemical analysis conducted at the end of the dosing period revealed no abnormalities attributable to the treatment.

Effect of ICFE on blood sugar level

Streptozotocin treatment will produce significant increase in serum glucose level with respective normal control group. The administration of ERLR 100mg/kg, 200 mg/kg and 400mg/kg and glibenclamide 10 mg/kg significantly reversed the increase in serum glucose concentration in Streptozotocin induced rats. The extract changes in the serum glucose level are shown in the table.

Diabetic mellitus (DM) is an endocrine disorder in which the glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic beta cells or because of inadequate release of insulin from the pancreatic cells of beta cells. The fundamental mechanism underlying hyperglycemia involved over population and decreases utilization of glucose by the tissue. In the presence study was observed that whether the EPLR extract have the effect of lipid profile, anti-oxidant system or not and in addition to its antihyperglycaemic action of STZ induced diabetic in albino rats.

Streptozotocin a beta cytotoxic induces diabetic in a wide variety of animal species including rats by selectively damaging the insulin secreting beta cells of pancreas i.p injection of STZ produces fragmentation of DNA of beta cells of pancreas which stimulates poly (ADP ribose and deflects NDA ultimately leading of destruction of beta cells and it is evidenced by clinical symptoms of hyperglycemia.

Dose dependent of the effect of the glibenclamide showed rapid normalization of blood glucose due to its insulin releasing effects.

In our present study there was a significant weight gain EPLR treated the diabetic rats compared with the normal control rats and this observation shows anabolic effect of the EPLR on body weight on the diabetic rats.

Hyperglycemia and insulin resistance both seem to have important in the pathogenesis of macro vascular complications. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The hyperglycemia in the diabetes might inhibit tissue repair in the macro vascular beds. In the present study of EPLR treated group shows hypoglycemic activity and it confirms the presence of the anti- diabetic activity. Sulfonylurea such as glibenclamide is often used as a standard drug in the STZ induced diabetic to compare to the efficacy of antihyperglycaemic compound. In study there was a significant elevation in blood glucose level in the diabetic control group as compared with normal animal. The ICFE treated group exhibited significant reduction of fasting plasma glucose level as compared to the diabetic control group. Over production of glucose by means excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus.

The most common observed lipid abnormalities in diabetics are hypertriglyceridemia and hypercholesterolemia. A marked increase in total cholesterol and decrease in HDL cholesterol have been observed in diabetics control rats. Insulin deficiency results in failure to activate the lipoprotein lipase thereby causing hypertriglyceridemia. There was a significant control of the level of serum lipids in EPLR treated diabetic rats. In diabetes, LDL carries cholesterol to the peripheral tissue where it is deposited, where HDL transports cholesterol from peripheral tissue to the liver and this aids its excretion. Hence increase in LDL is atherogenic. In our present study, there was a significant decrease in triglyceride LDL and T.C levels, where as there was a significant increase in the HDL level.

Diabetes is a common chronic ailment for which the patient has to take insulin to maintain the blood sugar level. It is interesting to see how the EPLR tackles this problem. It corrects the function of pancreas, stimulating it to produce insulin in the natural way, which in turns way to maintain the blood sugar level. EPLR revitalizes and rejuvenates the organs, the dysfunction of which is causing the disease. This bring back normal functioning of the organs. It is also maintaining the healthy state body. Since no artificial chemical are involved, it doesn't cause any side effects

8. SUMMARY AND CONCLUSION

The presented study is an attempt to investigate the effect of petroleum ether extract of EPLR on Streptozotocin induced diabetic in albino rats.

The Phytochemical study was screening showed the presence of tannins, carbohydrate, Flavonoids and reducing sugar which is responsible for the anti-diabetic activity.

The animals were induced with STZ at a dose of 55mg/kg intraperatoneal and the diabetic animals were treated with EPLR (100, 500, 1000mg/kg) for 28 days orally. The serum glucose, body weight lipid profile, liver glycogen were measured from the pancreas homogenate were measured which showed significant activity.

The finding of the presence investigation suggests the EPLR has potential for its evaluation as protective agents against toxicity induced by Streptozotocin.

Clinical assessments of EPLR determination of underlying mechanism of the protective effects in interesting topics requiring further study.

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